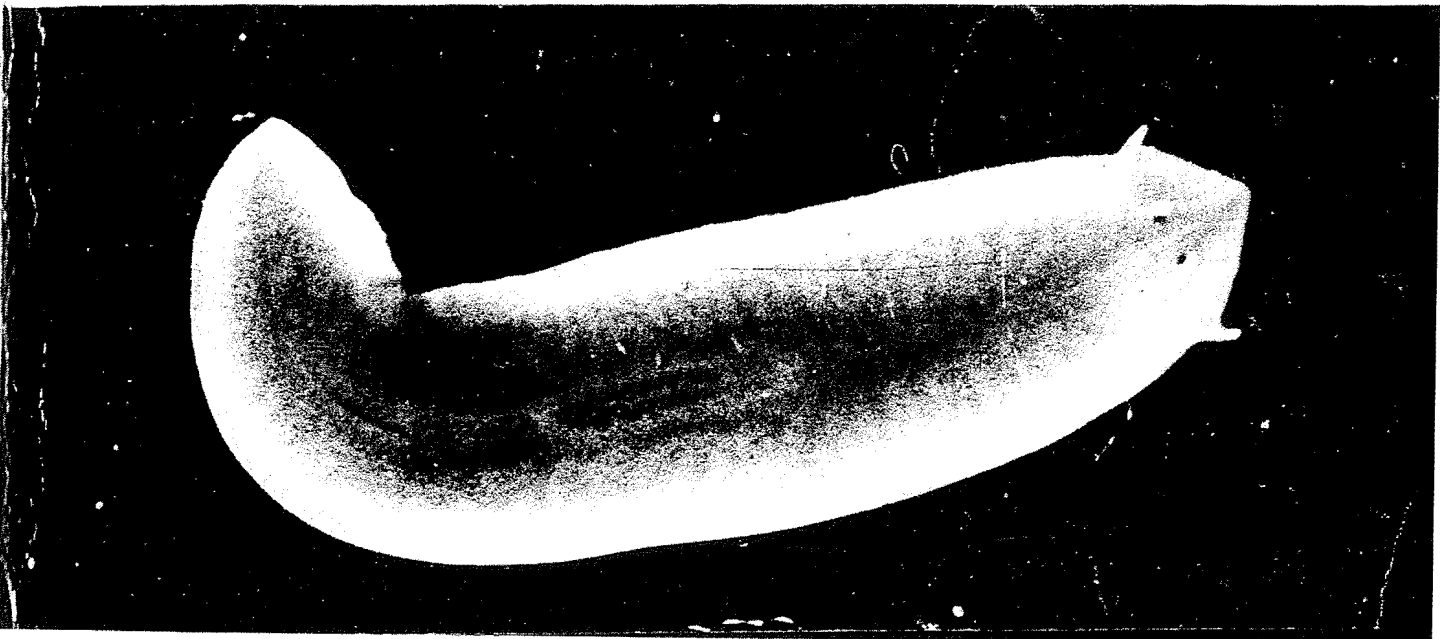


A MANUAL OF PSYCHOLOGICAL EXPERIMENTATION
ON PLANARIANS

Edited by

James V. McConnell



A MANUAL OF PSYCHOLOGICAL EXPERIMENTATION ON PLANARIANS

is a special publication of

THE WORM RUNNER'S DIGEST

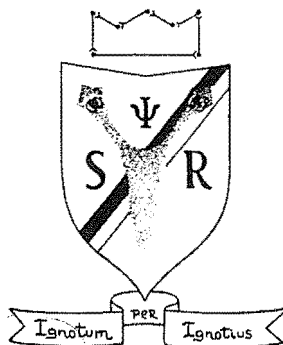
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DEDICATION

As measured in terms of man hours and money spent, science these days is a very expensive proposition. The facts that are presented in this MANUAL were purchased by hundreds of scientists who, individually and collectively, spent many weary years watching planarians both in nature and in the laboratory, and by the expenditure of hundreds of thousands of dollars in research funds. We cannot begin to name all of the people whose experimental findings are mentioned in this MANUAL, nor can we adequately thank all of the sponsors whose underwriting of various research programs made it possible for so many scientists to work with planarians. The least we can do, however, is to express our gratitude to those various agencies that have provided funds for the work of the Planarian Research Group during the past 10 years: The Office of Research Administration, the Department of Psychology, and the Mental Health Research Institute (all at The University of Michigan), the National Institute of Mental Health (Bethesda, Maryland), and the Atomic Energy Commission (Washington, D.C.). Without their muchly appreciated support, we never could have written this MANUAL.

INTRODUCTION

For a number of years now, we have received requests from all around the world for detailed information on "how to run worms." While we have done our best to respond to such inquiries in as great and specific detail as possible, it has not always been practicable to answer each letter personally. Usually we have suggested that anyone interested in performing planarian research go dig the facts out for himself, a laborious task at best, but necessary since to date no one has assembled a manual that would gather together in one place all of the odds and ends of information, the "rags and tatters" of methodology and procedure, that someone coming to the field for the first time would need to know. This MANUAL OF PSYCHOLOGICAL EXPERIMENTATION ON THE PLANARIAN is our own small attempt to meet what appears to be a continuing need. The MANUAL is addressed to the complete novice, for the majority of people who write to us for detailed information are high school students who have had but limited experience with animal experimentation of any kind. Thus the sophisticated experimenter may find that much of what is herein contained is superfluous for his purposes and perhaps too didactic for his tastes. The experienced investigator who has never handled planarians before would, however, be well advised to give this MANUAL as close a reading as possible--planarians are not merely miniature rats or cats, and the scientist who treats worms as if they were no more than "just another experimental animal" is in for some difficult times.

This MANUAL has seven main sections: The first is an article describing very briefly the history and importance of planarian research. The second section has to do with the acquisition, housing and care of various species of planarians, including a brief account of the animal's sensory capabilities. The third section is a detailed account of the methods and procedures for studying planarian behavior that seem to have been successful in the past and includes a brief reminder to all investigators of the sorts of data that should be included in any write-up of planarian research that one hopes to publish. The fourth section, written by Dr. Stuart J. Coward of the Department of Biology at the State University of New York at Buffalo, is an outline of procedures for performing planarian grafts. In the fifth section, Gary Davis presents a detailed account of the methodology developed by him and Dr. Bernard Agranoff (at the Mental Health Research Institute, The University of Michigan) for extracting RNA from whole planarians. In section six, Dr. Paul Shinkman and Leroy Vernon (of the Planaria Research Group, Mental Health Research Institute, The University of Michigan) describe the technique they developed for injecting micro quantities of RNA into intact planarians. The seventh and final section is a selected annotated bibliography of scientific articles having to do with planarians, taken from various past issues of the WORM RUNNER'S DIGEST and put together by Dr. Margaret Clay and Miss Ursula Mosler (of the Planaria Research Group). This bibliography includes, to the best of our knowledge, all pertinent and important references to planarian research except those that have been published in the DIGEST itself. Digest articles are listed chronologically immediately following the bibliography. The serious "worm runner" will surely wish to obtain a complete file of the back issues of this now notorious journal.

Experiments on planarians may often seem deceptively "simple-minded" to the casual observer. Let said casual observer beware: planarians are among the most obstinate, ornery, difficult animals to work with that one could imagine. They can also be highly rewarding as experimental subjects. But anyone who believes that he can "throw together a quick and dirty worm study over the weekend" is likely to be shocked to learn that this type of scientific approach is even less likely to yield valid results with planarians than with other animals. The most salient virtue that a worm runner

can possess is patience.

In the past five years, several thousand high school students throughout the United States have used planarians in their science fair projects; it is likely that several thousand students will do likewise in the coming five to ten years. Many high school students in the past have written us requesting that we send them apparatus, worms, free copies of the DIGEST, etc. This is to inform all and sundry students (of any age or status) that we cannot supply anyone with free worms, free apparatus, nor even (in the majority of cases) with free personal advice. A good share of becoming a scientist is learning how to solve one's own problems, with frequent recourse to the scientific literature. We have listed elsewhere in this manual information on the procuring of planarians and on the construction of experimental apparatus--this, then, is the limit of our responsibility. Were we to answer each inquiry individually, we would end up spending more time writing letters than in doing research ourselves. And planarian research does take time--a good experiment will require at least four to six months of hard effort. Nor can you expect us to sit down and tell you in detail what sort of research has already been done in the past and help you design a new and exciting line of investigation that will assuredly win you a prize. You must expect to read the literature yourself and design your project yourself, for otherwise the whole experience would have little real significance for you. We do hope that you will let us know what sort of work you have done and what your results were, but we regretfully announce that we cannot usually give you much assistance beyond that contained in this MANUAL.

We have done our best to make the information in this MANUAL as correct and as up-to-date as possible, but we do not pretend to be omniscient even where planarians are concerned. The field of flatworm research is in constant flux, and as new studies are published and as new techniques are devised, some of the information contained herein may become obsolete or may even be proved to be incorrect. The ways of worm running are subject to change without prior notice! It will be up to the reader to check all references that appear after the MANUAL has been printed and to revise his procedures and conclusions accordingly.

One final note: Anyone who has had experience training other animals (such as rats) will find that the training of planarians is several orders of magnitude more boring than is the handling of rats or other higher animals. The confirmed rat runner may find such a statement hard to believe, but a few hours spent with planarians will surely convince him of its truth. Nonetheless, when all is said and done, planarians are rather wonderful and challenging beasts whose rather special biological and psychological talents and properties can make them fascinating subjects for scientific investigation. The worms will provide the fascination--it is up to you to provide the science.

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May 1, 1965
Ann Arbor, Michigan

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Section I

THE MODERN SEARCH FOR THE ENGRAM*

James V. McConnell, Ph. D.

Man can learn more, and remember better, than any other animal--indeed his prodigious memory is one of the chief attributes that sets man apart from the rest of the animal kingdom. That a ninety-year-old man may recall the halcyon days of his youth with surprising clarity is a commonplace occurrence; yet, when one stops to think of it, how can this miracle be accomplished? How can a fragment of conversation heard 40, 60 or even 80 years ago still be remembered word for word after such a length of time? What kind of recording does the brain make that allows it to play back those dead voices years afterwards? Of what are our memories made, and where and how are they stored inside our bodies? Indeed, does it make sense to speak of "memory storage" at all as if a memory were a physical quantity of some kind that could be stashed away in a cupboard or a refrigerator? The search for answers to these questions is one of the most challenging and fascinating undertakings in the biosciences today. But to understand the viewpoint that modern psychologists and biochemists take towards memory research, we must first place the subject in its proper historical perspective.

The early Greek philosophers believed that man was born with a mind like a blank tablet and that the fingers of experience "wrote" on the tablet much as a secretary copies down dictation in her notebook. When we "remembered," then, we merely searched through the mind's library of old notebooks until we found what it was we were hunting for. And indeed, when we are attempting to recall an old friend's name, or a lost fragment of poetry learned years ago, it does seem as if our minds flip through a catalog of past events. But such a viewpoint towards remembering is a limited one at best. To begin with, some memories are automatic--we don't have to "search" for them at all, for the external environment triggers off the proper bit of recall almost mechanically. In the second place, much of our remembering takes place at a totally unconscious level--we don't have to tell our hands and feet to press on the clutch and shift the gears in our automobiles; our feet and hands make the proper motions without our consciously directing them, almost as if "they had a mind of their own." The mentalistic "notebook" idea of memory, a viewpoint that science espoused for almost 20 centuries, was finally abandoned because it taught us precious little about memorial processes that common sense hadn't told us anyhow.

The real revolution in the study of memory began in Germany in the middle of the 19th Century with the work of Hermann Ebbinghaus, who performed the first really scientific experiments on how men learned. Ebbinghaus' monumental handbook, Ueber das Gaeedechnis, is still considered a classic by modern psychologists. But Ebbinghaus was interested in the external conditions that made remembering easy or difficult; his theoretical explanations of why men remembered as they did were tinged with mentalism. He did not try to look inside the human body to see what sorts of changes took place inside whenever someone learned.

The behaviorist revolution, which began in America with the work of John B. Watson, was the next step forward. Watson saw very clearly that man often tends to attribute to himself almost magical mentalistic properties which he denies exist in animals. Desiring to get rid of magic and substitute science in its place, Watson espoused the study of animal behavior since one could be much more objective about the way a white rat learned a maze than, say, about the way a pretty girl learns a popular song. But Watson's way of tossing

out the magic of mentalism was to deny that anything of importance took place inside the body when a person learned. In Watson's theory, all animals became "empty organisms." If one but knew all of an animal's past behavior, and if one knew the present stimulus conditions the animal faced, one should be able to predict the animal's behavior completely. Watson then looked for correlations between stimuli and responses without ever taking into account the organism that sensed the stimuli and made the responses. The purely behavioristic viewpoint was a powerful one for, under the guidance of such brilliant theorists as B. F. Skinner at Harvard, we learned a great deal about how to control the behavior of organisms in many rather complicated situations. But the behaviorists' stimulus-response correlations have but limited generality, they predict very poorly what animals will do when faced with new stimuli, and the correlations tell us nothing at all about why memory takes place, why some animals are better at remembering than others, why we forget when we're shocked, or a dozen other things.

Learning and remembering are such natural things to most of us that perhaps it is difficult for us to realize that these processes must have a physical representation inside our bodies. We see ourselves, like behaviorists, from the outside in. We do not sense the flow of blood through our veins, the movement of fluid in our spinal cord, nor the pulsation of electrical energy coursing through our nervous systems. Yet these processes exist--if ever they stopped, we would die almost at once. Life is a physiological process, and memory is a part of life. When we learn, there must be some actual, identifiable alteration in some part of our body; otherwise, we could not, at some later date, reconstruct with mere nervous energy that environmental condition we had experienced in the past.

At about the same time that the behaviorist revolution was beginning in America, when John B. Watson was telling his students that "mind didn't exist," the noted Russian physiologist, Ivan Pavlov, was telling his students that "mind" must be translated into neurological terms to make sense. For Pavlov, the cerebral cortex, that massive envelope of nerve cells covering the entire brain, was the seat of learning. Pavlov thought that learning was a process, a rechanneling of the flow of nervous excitation from an old pathway into a new. What the structural changes were in the nervous system that caused the change of pathways was something neither Pavlov nor the neurophysiologists who followed him were able to determine. Physiological psychologists have, for half a century now, attempted to determine just exactly what the neurological changes are that take place within an organism when it learns; but for all their delicate and sensitive electronic gear, their surgical insults and their probing microelectrodes, they have failed almost completely. It is this failure of the physiologists to specify precisely where or how memory storage takes place, coupled with our recently-gained knowledge of the biochemistry of the cell, that has led many of today's scientists to search for the "locus of memory" at a chemical rather than a purely physiological level.

It was in 1904 that a scientist named Richard Semon made a radical assumption. Suppose, he said, that each stimulus that an organism experiences leaves a discrete material trace of some kind within the organism's nervous system. Semon went further--this material trace, which he called an "engram", he saw as being perhaps a chemical rather than a physiological entity. Each unique stimulus encountered by an organism, each thing that it learned, was to leave its own particular trace, its own unique engram. Many years later, the great American psychologist, K. S. Lashley, devoted an enormous amount of research time to what he called "the search for the engram." If the engram was unique, special, particular, then one ought to be able to localize the engram for a particular bit of learning in a specific place within the nervous system. Lashley taught

rats and other animals to solve various problems, then cut out huge portions of the animals' brains hoping to remove the engram along with the ablated tissue. Lashley never located the engram, for his animals showed only "relative" forgetting. If he removed 50% of their cortex, they forgot about half the problem; if he removed but 25% of the cortex, they remembered approximately 75% of what they had learned. These results led Lashley to conclude that a single memory was represented in the brain by a diffuse process of some kind rather than being stored in one discrete locus. What Lashley never realized, of course, was that an animal that learns even a "simple" problem actually learns thousands and thousands of things about the environment in which the experimenter puts it. A rat in a simple maze learns not only that it must turn right if it is to get to food, but learns also that the maze is of a certain color, certain texture, of a certain height, smell, width and length, that the food is of a certain type and certain quantity, and a thousand other things. The rat must learn to "trust" the experimenter (that is, to become habituated to the experimental situation) or it will not eat, will not learn. All of these thousands of individual micro-memories go to make up a single "learning experience." Some of the memories are visual, some auditory, some kinesthetic, some olfactory; obviously the engrams would be widely scattered in various parts of the brain, and if one removed portions of the cortex at random, one would expect to obtain the results that Lashley did. And Lashley was searching for a neurophysiological rather than a biochemical locus of learning; apparently it never occurred to him that the engram might possibly be stored molecularly, as a complete entity, within a single cell.

Let us assume for the moment that each time an organism learns, a complicated biochemical change of some kind takes place within at least one nerve cell in the organism's brain. What chemicals might be involved, what chemicals might act as storage mechanisms? In the late 1940s, Ward Halstead, a psychologist at the University of Chicago, theorized that the engram might exist as a molecular change within a single cell. It was not until almost a decade later, however, that the Swedish biologist Holger Hydén undertook a series of experiments that first implicated ribonucleic acid (RNA) as being the "memory molecule." The genes found in every cell are composed of a complicated molecule known as DNA, which acts as the Commander-in-Chief of all physiological processes in the cell. DNA directs the internal life of the cell by manufacturing RNA, a closely related molecule, which passes from the nucleus into the cytoplasm of the cell where it directs the synthesis of proteins (among other things). DNA contains the "genetic code." When the sperm and egg from a pair of white rats unite, the resulting embryo develops into a baby rat rather than into a baby elephant because the DNA in the rat sperm and egg contains a different "code" than the DNA found in elephant sperm and eggs.

From one point of view, then, we may say that DNA stores an organism's "ancestral memories" in coded form; that is, DNA "remembers" what an organism's progenitors were like. Professor Hydén theorized that RNA might encode or "remember" an organism's own personal memories, that RNA might well be the "tablet" on which the fingers of experience wrote (by changing the chemical "code" carried by the RNA molecule). To test his theory, Hydén performed experiments with rats and rabbits. First, Hydén developed a beautiful technique for taking large single cells from the nervous system, cutting them open individually by hand with an incredibly tiny scapel and scraping out the protoplasm inside each cell. With microanalytic techniques, he was then able to measure rather subtle changes in the RNA found in these cells. Then he took two groups of rats to work with. The first group was trained to balance on a taut wire in order to reach food; the second group was given passive exercise but did not learn the balancing trick. Hydén found that the gross amount of RNA increased markedly in cells taken from both groups of animals, but qualitative changes in the RNA (that is, apparent changes in the "code"

itself) took place only in the nuclear RNA taken from the trained animals. Hydén later discovered that the gross amount of RNA in the human brain increased from birth until age 40, remained at a relatively constant, high level until age 60, then rapidly began to decline. These findings led Hydén to speculate that memories were stored by means of physical changes in the RNA molecule itself. Hydén's theory, which has actually never been proved or disproved, has sparked much of the recent but growing interest in the biochemistry of memory and has prompted dozens of other scientists to undertake related experiments on a wide variety of organisms ranging from the flatworm up to the human.

The Planarian as an Experimental Animal

Anyone at all familiar with the planarian, or common freshwater flatworm, might well wonder why a psychologist would pick this animal to experiment with. Seldom growing to more than an inch in length, it is a fragile creature that inhabits streams, ponds, lakes and rivers throughout the world. The planarian is often difficult to maintain for long periods of time in a laboratory situation and its nervous system is exceedingly primitive--so primitive indeed that its ability to learn even the simplest of tasks might be questioned on purely theoretical grounds. Yet the flatworm does have a rudimentary brain, its behavior is much more complicated than one might imagine at first glance, and the planarian possesses certain other rather special characteristics that make it a most useful tool in the search for the biochemical correlates of memory.

The first time that I personally encountered a flatworm was in 1953, when I was a graduate student working towards my doctorate in experimental psychology at the University of Texas. At that time, psychologists in general were just becoming aware of the neurophysiological learning theories of D. O. Hebb, the Canadian psychologist, and of Sir John Eccles, the Nobel Prize winning physiologist from Australia. These two scientists had speculated that learning was a matter of a physiological change at the synapse--that is, whenever an organism learned, some structural alteration must take place in its nervous system, probably at the junction point between two nerve cells which is called a synapse. Obviously, if Hebb and Eccles were correct, an organism would have to possess synapses in order to learn. Robert Thompson, then a fellow graduate student at Texas and now a Professor of Psychology at Louisiana State University, suggested to me that since the flatworm is the lowest animal on the phyletic tree that possesses the type of synapse Hebb and Eccles had in mind, we might test their physiological learning theories by attempting to train a planarian. And so, working together, we set out to do just that.

The first scientist to attempt to train planarians was probably the Dutch biologist P. Van Oye who reported in 1920 that he was able to teach these animals by means of a rather unusual training technique to crawl down a thin metal wire to get to food. (Van Oye's paper was published in Dutch, however, and we did not discover it until many years after we had begun our own studies.) In 1929, the American psychologist H. B. Hovey trained a marine flatworm (*Leptoplana*) to reverse its normal response to light by punishing it every time it moved when a light came on. And in the 1930s, two German scientists, F. Dilk and H. Soest, attempted a crude form of classical conditioning with planarians. None of these latter-named studies satisfied Thompson and me, however, for they all lacked crucial control groups needed to convince us that learning had actually taken place. Therefore we set up our own experiment, complete with what we believed to be the necessary controls.

A modified version of our first training apparatus appears elsewhere in this manual. It consisted of a foot-long, half-inch-wide plastic tube that could be filled with water.

Electrodes mounted at each end of the tube allowed us to pass an electric current through the water. A pair of lamps suspended above the trough provided the light which served as the conditioning stimulus. When a planarian was crawling smoothly along the trough and we turned on the electric current, the animal would always give a vigorous contraction of its entire body. We trained the animals by pairing the light with the shock. First we turned on the light for 2 seconds; then we turned on the shock for an additional second, then we turned off both the light and the shock. At the beginning of training, the worms responded to the light (before the shock came on) no more than 25% of the time. After we had paired the light and the shock for 150 trials (all given in a period of 2 hours or so), the worms responded to the light 50% of the time, just as if they had now learned that the light was a signal that shock was coming. A group of animals given 150 trials of light without shock showed the expected phenomenon of habituation--that is, they responded less and less often to the light during training. Another group of worms, given 150 "shock-only" trials also showed a decreased sensitivity to occasional test trials of light (which was never paired with the shock). Since the increased responsiveness in our experimental animals was quite statistically significant, and since our various control groups showed us that this increase was not due to sensitization, we were convinced that we had shown clear-cut classical conditioning in planarians. The article describing our research was published in the Journal of Comparative and Physiological Psychology in 1955. In 1956, when I became an Instructor at The University of Michigan, I set up my own laboratory here and continued the planarian research.

To most people, I suppose, the flatworm is famous not because it is the lowest animal with a rudimentary brain and a true synaptic type of nervous system, but rather because it possesses enormous powers of regeneration. The species of planarian we most often use in our experiments is Dugesia dorotocephala. If one cuts this animal in half transversely, the animal does not die; rather, the head grows a new tail and the tail grows a new head, and each regenerated piece will eventually become as large as the original organism. Indeed, under the best of conditions, one may cut this animal into as many as 50 pieces and each section will regenerate into an intact, fully-functioning organism.

It had occurred to Thompson and me that it might be interesting to train a planarian, then cut it in half and test the regenerated sections to see which portion (if any) would retain the memory of the training; Thompson and I never got to this study at Texas, however. But once my laboratory was set up at Michigan, two of my students (Allan Jacobson and Daniel Kimble) and I undertook that very study.

Giving planarians 150 trials all in a period of 2 hours is not a very effective way of training them; flatworms, like humans and rats, will usually learn much better if the trials are stretched out over a period of several days. By spacing the trials, we were able to get each of our worms to respond to the onset of the light at least 92% of the time. When each animal reached this criterion, we cut it in half and allowed it to regenerate for a period of a month. Then we retrained each regenerated half to the same 92% response level to determine if it learned faster the second time than the first (this so-called "method of savings" was one of the pioneering methodological contributions of Hermann Ebbinghaus). We had expected the heads to remember at least some part of what they had been taught--after all, the head section retained the brain and most of the nervous system of the original animal. Our expectation was confirmed--the head regenerates remembered just as well as did planarians that were trained but not cut in half. But what of the tails? They had to regrow an entire new brain and replace most of their vital organs during the month's regeneration. How could one expect a regenerated tail to remember anything at all? The answer is, of course, that one could not expect the tails to remember at all--but they did.

In fact, many of the tails showed almost perfect retention of the original training! It seemed as if their new brains were created with the old "learning" already "wired in" much the same way that an innate behavior pattern ("instinct") is "wired in" from birth. Furthermore, we soon found that if we cut the trained worm in thirds, or even fourths, each regenerated piece would show significant retention of the memory.

Our "retention following regeneration" results, published in the Journal of Comparative and Physiological Psychology in 1959, were at first considered somewhat unbelievable. Since then, however, the study has been replicated in dozens of different laboratories all around the world. The results are apparently valid--but the nagging question remains, how could the tail remember anything at all?

As I thought about this question back in 1958, it occurred to me that the "engram" must be stored throughout the planarian's body, not just in the animal's brain, and that the storage mechanism probably was chemical in nature. Indeed, it seemed obvious that the same engram was probably duplicated hundreds of times throughout the animal's body, being stored within hundreds of individual cells. But what was the chemical (or chemicals) involved in memory formation? It was at this time that I became aware of Hydén's RNA theory of memory. Now, the cell that I thought was probably involved in memory storage in the planarian was the neoblast--a primitive, undifferentiated cell that can migrate anywhere in the animal's body and which has the property of being able to develop into any other type of cell whatsoever. According to such noted scientists as Prof. Etienne Wolfe of the College du France and Prof. H. V. Brøndsted of the University of Copenhagen, the neoblast is the cell which is primarily responsible for the planarian's powers of regeneration. When the animal is cut in half, the neoblasts migrate to the site of the wound and form a bud or blastema from which the missing parts of the animal are regenerated. The neoblast is also very rich in RNA, a fact which seemed to support my hypothesis that this primitive cell might act as the site of the engram in planarians.

The first experimental test of the RNA theory of memory with planarians was, however, performed by Prof. E. Roy John and his student, William Corning, at the University of Rochester. John and Corning reasoned that if they would somehow destroy or alter the RNA in a trained planarian's cells, they would also destroy or alter the memory itself. Therefore, in 1960, they trained flatworms using the light/shock conditioning technique we had developed and, once the animals had reached criterion, cut them in half. Some of the animals were allowed to regenerate in the usual pond water; others were forced to regenerate in a weak solution of ribonuclease (an enzyme which hydrolyzes or breaks up RNA). As they reported in *Science* in 1961, both heads and tails which regenerated in pond water showed the usual retention, as did the heads which regenerated in ribonuclease. The tails which regenerated in the ribonuclease, however, showed almost complete forgetting of the original training, much as if the enzyme had somehow erased the blackboard on which the memory was written. A recent study by Fried and Horowitz at UCLA indicates that much the same result obtains when one injects ribonuclease directly into the body cavity of trained planarians. Ribonuclease is a very powerful destructive agent, however, and there is still some question whether these studies show that the ribonuclease affected specifically the memory storage or whether the "forgetting" was due to a more general, debilitating effect on the animals' health.

While Corning and John were working with ribonuclease, we attacked the RNA hypothesis from rather a different direction. It had been known for a great many years that planarians lacked any "immune" reaction, and that one could often graft a small portion of one animal's body onto the body of another planarian. This non-specificity of planarian tissue

suggested that we might be able to transfer engrams from one animal to another if we could somehow get chemicals from a trained animal into the body of an untrained planarian. Our first attempts at grafting large portions of one animal onto another were not particularly successful, however, and so we turned to what was a simpler but perhaps a more spectacular type of tissue transfer, that of "cannibalistic ingestion."

Many species of planarians, when hungry, cannibalize quite readily. The species we have used most often, *Dugesia dorotocephala*, is a particularly voracious cannibal. Beginning in 1960, my students and I conducted a series of experiments involving what we now call "the cannibalistic transfer of training" in planarians. First we trained some "victim" worms to criterion (using the now-standard light/shock conditioning technique). We then fed the trained victims to starved, untrained cannibals. At the same time, a set of untrained "victim" worms was fed to a different group of cannibals. Both sets of cannibals were then given light/shock training. The results were clear-cut: the cannibals that had ingested "trained" victims were, on the very first day of training, significantly superior to the cannibals that had eaten untrained animals.

We repeated the cannibalism study four times over, employing each time a "blind" testing technique (in which the person actually doing the training does not know the prior histories of any of the animals he trains). Each time, as Barbara Humphries, Reeva Jacobson Kimble and I reported in the Worm Runner's Digest in 1961 and in the Journal of Neuropsychiatry in 1962, the results were essentially the same. By early 1965, some type of cannibalistic transfer in planarians had been reported by at least 10 other university laboratories (and by hundreds of American high school students working on what we call "Science Fair" projects).

Although we now jokingly refer to these studies as confirming what we call our "Mau Mau" hypothesis, and although they have been widely replicated, one obviously should not generalize these results to the human level too readily. To begin with, the planarian has a very simple digestive system; it lacks a true stomach and does not secrete the massive array of acids and enzymes necessary for digestion in higher organisms. RNA would surely stand little chance of being absorbed in an unaltered form in higher organisms. And, in the second place, we still do not know just what it is that actually gets transferred via ingestion, whether the cannibal acquires a specific memory or perhaps just a general response tendency, or perhaps both. However, there is a growing body of evidence suggesting that, at least under certain conditions, it is a particular and specific engram that gets passed from one animal to another.

No matter what it is that gets transferred by ingestion, however, we are still left with the problem of finding what the transfer mechanism itself is. If RNA were really involved, we obviously should be able to achieve the transfer if we trained some planarians, then extracted the RNA from these animals and injected it into untrained planarians. We began work on this problem in 1962 and, with the support of grants from the U.S. Atomic Energy Commission and the National Institute of Mental Health, are still continuing our studies today.

Our first experiment was a most crude undertaking. Prior to our work, there had been little need to extract RNA from living tissue for any purpose other than analysis. The extraction techniques usually employed then tended to degrade the RNA rather badly and typically gave quite small yields. We needed to get as much RNA as possible from each animal and wanted it to be broken up as little as possible by the extraction process. We soon found that, employing the crude techniques at our command, we needed the tissue

from several hundred trained planarians in order to extract sufficient RNA to inject into 10 untrained animals! Since it takes from 10 to 20 hours (spread out over several days) to train one worm, and since we needed at least 500 animals in each of several groups in order to perform the experiment, it became clear to us that we could not give the animals individual, personalized training. Rather, we were forced to put 100 or more planarians in the training apparatus at the same time with the hope that after the "group" had been given several hundred trials, most of the animals would have learned something. Of course we had no way of telling just how much any of the animals had learned.

In our first experiment, we had some 500 experimental planarians given the usual light/shock training (in a group), plus 500 animals in each of several control groups. After training was complete, we extracted the RNA from each group and injected it into untrained planarians and then tested the injectees in our usual "blind" fashion. The animals injected with RNA from the trained planarians were significantly better than any of the animals injected with "control" RNA. A repetition of the study with a different set of control groups yielded essentially the same results. In both cases, however, the differences (while statistically significant) were not as great as was the case in the cannibalism studies. Perhaps the surprising fact is that, considering the crudeness of our methods, we got any significant results at all! We have spent the past two years or more attempting to improve both our extraction methods (our first RNA was badly contaminated with polysaccharides and other cellular materials) and our means of training large numbers of animals at a given time. At the moment, it seems possible that we will be able to undertake an adequate replication of this work by the summer of 1965. Meanwhile, I am pleased to report that Fried and Horowitz, working in the Department of Psychology at the University of California, Los Angeles, were apparently able to confirm our findings this past year using an entirely different extraction technique than the one we have been developing.

In addition to the studies mentioned above, there are several other experiments which seem to link RNA with memory formation. Two other groups of scientists working here at the Mental Health Research Institute at Michigan have reported data which are pertinent to this problem. Prof. Ralph Gerard and his associates found that injections of 8-azaguanine (which inhibits RNA synthesis) increased the time required for "fixation of experience" in rats and that injections of malononitrile (which stimulates RNA synthesis) did just the opposite, while Agranoff and Klinger recently reported that puromycin (which interferes with the synthesis of RNA in brain cells) injected intracranially into goldfish produces impairment of memory for a shock-avoidance response in these animals. Flexner and Flexner, at the University of Pennsylvania, had earlier observed that puromycin had much the same effect on avoidance learning in mice as Agranoff and Klinger report in goldfish. And Dingman and Sporn, at the National Institutes of Health in Bethesda, Maryland, report that when they injected 8-azaguanine into the brains of rats being trained in a maze, the injectees made twice as many mistakes as did control animals.

The fact that substances which facilitate or inhibit production of RNA in vivo also speed up or retard learning does not, however, mean that RNA is the "memory molecule," since these effects might well have been due to an interference with protein synthesis or some other cellular function. However, Cook, Davidson, Davis, Green and Fellows have reported that when they gave brain injections of yeast RNA to rats, the animals learned an avoidance habit significantly faster than did control animals. Pickett, Jennings, and Wells, at Occidental College in Los Angeles, have recently reported much the same thing for planarians trained in mazes. And Cameron and his colleagues at McGill University in Montreal have found that administration of yeast RNA to aging patients sometimes retards

or even reverses the memory deterioration which often plagues the elderly. Improvement is most marked in arteriosclerotic patients, less so in presenile or senile patients. Montanari, Cutolo and Mazzoni in Italy have reported similar results using a mixture of nucleotides rather than RNA. (Unfortunately, most RNA now available commercially tends to induce fever in human subjects; until a sure method for removing the pyrogens from yeast RNA is discovered, Cameron's findings will probably have more theoretical than practical significance.)

At the moment, it seems safe to conclude that RNA is intimately involved in the storage of memories, but it is obviously far too soon to conclude that RNA is indeed the "memory molecule" itself. As Dingman and Sporn have pointed out, it is likely RNA is but one small part of a complicated chain of cellular processes that, taken all together, functions to store our "remembrance of things past." It is my own opinion that RNA may play two distinct and somewhat different roles in memory formation. First, since Hyden's experiments indicate that RNA is quite sensitive to changes in the neuron's external environment, it may be that incoming sensory signals (that is, an organism's experiences) are "coded" first of all by physico-chemical changes in RNA molecules. The altered RNA would then produce different types of proteins than before the message was encoded and the neuron would function differently than before. But second, the "transfer" studies on planarians suggest that RNA may occasionally serve to transport coded information from one part of the body to another. Quite recently, Bonavita and his colleagues have reported that mononucleotides can probably pass through the blood-brain barrier in mammals. If RNA can enter into and exit from the blood stream in the same fashion, it is quite possible that Nature uses RNA as her means of transferring engrams from one point in the nervous system to another, even from one side of the brain to the other. If an engram formed in one part of the brain could be spread via RNA to distant parts of the nervous system, it is no wonder at all that Lashley was unable to find the "locus" of the engram even by extirpating very large portions of the cortex.

Let me conclude by pushing my speculations to perhaps an incredible limit. If "coded" RNA is indeed Nature's "transfer agent," and if we can discover what that code is, we may eventually be able to implant some types of knowledge directly into a human brain by injection of suitable "memory molecules." Whether or not the day of "instant learning" ever dawns remains to be seen. But it is certain that the vistas opened by research on the biochemistry of memory will be fascinating ones for all of us to explore.

*Reprinted from "n+m" (published by C. F. Boehringer & Soehne GmbH of Mannheim), in press.

Section II

ON THE PROCURING AND THE CARE OF PLANARIANS

James V. McConnell

Introduction

This Manual will concern itself chiefly with the behavior of planarians, hence little reference will be made to the extensive literature on the zoology of these animals. The scientist who expects to perform meaningful psychological studies on planarians, however, will do well to familiarize himself with what goes on inside these animals. No better source for such information can be found than the works of Hyman (see the annotated bibliography at the end of this Manual for complete references). In brief, planarians belong to the phylum Platyhelminthes, the order Turbellaria, and the family of triclads. Members of this phylum are the simplest animals to possess bilateral symmetry, a rudimentary brain which is located in the front end of the animal (anterior encephalization), and the complicated, synaptic-type nervous system found in all higher phyla. Thanks to some very recent work by Jay Boyd Best (Colorado State), we now know that their brains, although quite small in size, are really much more complicated and highly developed than was thought to be the case when Hyman wrote her classic text. Planarians commonly do not have a mouth, but rather eat by means of a food tube or pharynx which is extruded from the center part of the under side of the animal's body whenever food is present, or whenever the animal is traumatized. The planarian has no noticeable circulatory system, but the stomach runs from the tip of its head to the tip of its tail. Digestion in the planarian is primarily intra- rather than inter-cellular; planarians do not secrete the array of acids and enzymes which aid higher organisms in their digestion of food. Planarians, when sexual, are hermaphroditic; during copulation, cross-fertilization occurs and both planarians produce cocoons containing about 16 eggs. The exact characteristics of these animals vary markedly from one species to another.

SPECIES

Planarians are found widely distributed throughout the world in oceans, ponds, streams and rivers, and on the land. The marine (salt water) species are quite common, but their behavior has been little studied. The classic research with marine flatworms was performed in the late 1920s by Hovey, who demonstrated that an innate reaction to light could be suppressed if he repeatedly tapped the animals on their snouts. The land planarians, sometimes brightly colored, often grow to be 40 to 50 inches in length. While found chiefly in hot, humid climates, land planarians can occasionally be discovered hiding under flower pots and other receptacles in hot houses and botanical gardens even in the far north. Land planarians, unlike their aquatic cousins, are chiefly herbivorous. The only two studies on the learning in land planarians with which I am familiar are by Noel Weeks and by Barnwell, Peacock, and Taylor. Both studies appeared in the October 1964 issue of the Worm Runner's Digest.

Several of the more common type of American freshwater planarians are shown in the accompanying photographs. Dugesia tigrina is perhaps the most common freshwater



Fig. 1. Dugesia tigrina



Fig. 2. Dugesia dorotocephala



Fig. 3. Phagocata gracilis



Fig. 4. Phagocata oregonensis

planarian and is widely distributed throughout the United States. It seldom grows to more than 20 mm. in length. D. tigrina regenerates fairly well, undergoes spontaneous fission readily, is somewhat less cannibalistic than D. dorotocephala, is more sensitive to x-irradiation than D. dorotocephala, and is very lightly colored. Indeed, D. tigrina is considerably more "transparent" than is D. dorotocephala (or any of the other more common types of planarians found in the United States). Reproduction is chiefly asexual; we have seldom encountered a sexual specimen of D. tigrina. The undersurface of D. tigrina is typically colorless and appears to have the consistency of nearly transparent gelatin.

Dugesia dorotocephala has a much darker, less spotted color pattern than D. tigrina. The under surface is often heavily pigmented and seldom has the "gelatin" appearance common to D. tigrina. D. dorotocephala is found chiefly in springs and running streams but is nonetheless, fairly common in the United States. D. dorotocephala is much larger than D. tigrina as a rule; by overfeeding the animal, we have gotten specimens well in excess of 50 mm. in length. Sexual sub-species of D. dorotocephala are quite common, and can often be induced to mate in the laboratory. Such mating is, however, usually seasonal and sporadic; we have, however, encountered one sub-species of D. dorotocephala taken from a spring in Oklahoma that remains sexual throughout the year and mates continuously. The sexual sub-species of D. dorotocephala do not regenerate as well as the asexual. The mature, sexual specimens undergo spontaneous fission but rarely. D. dorotocephala is a voracious cannibal and will cannibalize even when decapitated. Indeed, I have seen a freshly decapitated specimen move forward and attempt to cannibalize its own head immediately after decapitation took place.

A third fairly common type of freshwater planarian found in the United States is Phagocata gracilis, which can readily be differentiated from Dugesia by its much darker pigmentation and the squareness of its head. D. tigrina and D. dorotocephala have but one pharynx; P. gracilis has many pharynxes. Its much darker pigmentation protects it from x-irradiation to a greater extent than is true with D. tigrina and D. dorotocephala. P. gracilis regenerates poorly, if at all; we have never been able to induce sexual behavior in this species in our laboratory. P. gracilis cannibalizes poorly, if at all. This species typically attains lengths intermediate between those of D. tigrina and D. dorotocephala.

A fourth fairly common type of planarian is Cura foremanii. Pigmentation in this species is typically darker than in Dugesia, although the two species resemble each other superficially. C. foremanii typically grows to about the same size as D. tigrina, however C. foremanii does not regenerate as well as D. tigrina since it is a sexual species.

For descriptions of other common planarians, see Hyman.

Most of the research in our laboratory has involved D. tigrina, D. dorotocephala, and P. gracilis. In choosing a species for behavioral experiments, one must usually take into account the type of training to be done in order to pick a "good" experimental animal. D. tigrina clings to surfaces tenaciously, and moves distinctly more slowly than does D. dorotocephala or P. gracilis. D. tigrina seems much less "excitable" in its movements than the other two species. We therefore tend to prefer to use D. tigrina in studies on classical conditioning but not in studies involving maze training--its slow, stately, unexcitable mode of locomotion, ideal for conditioning experiments, makes it a frustrating animal to observe in mazes or open field experiments. Obviously, if one wishes to study cannibalism or regeneration, one picks a species other than P. gracilis.

Our own studies on the effects of x-irradiation on regeneration in planarians show

that D. tigrina has a LD₅₀ (30 days) of around 800 roentgens (that means that when the animals are given an acute dose of 800 roentgens, half of the animals die within 30 days following the irradiation). D. dorotocephala has an LD₅₀ (30 days) of approximately 1000 roentgens, while P. gracilis has an LD₅₀ (30 Days) that lies between 1200 and 1400 roentgens. Further research indicates that D. tigrina and D. dorotocephala can be given chronic doses of 200 roentgens per week and will survive indefinitely, even though they accumulate dosages well in excess of 20,000 roentgens.

Planarians contain a type of primitive, undifferentiated cell called the neoblast, which apparently gives them their enormous powers of regeneration. According to Professor Etienne Wolfe, the neoblast is found scattered throughout the body of the planarian; when the animal is wounded, the neoblast migrates to the locus of the wound where it forms a bud or blastema of regeneration from which the missing parts of the animal regenerate. The blastema therefore has the power to differentiate into any other type of cell. According to Professor H. V. Brøndsted, as many as 30,000 neoblasts may be contained in a single planarian's body. For additional information on planarian regeneration, see the articles in that section of the annotated bibliography in section seven of this manual.

According to Hyman, sexuality can be induced in some planarians by putting them in a cold place for a few days before returning them to the normal warmth of the laboratory (70° F.). We have seldom found this technique to be of great value. While some species may be induced to mate in the laboratory, it has been our observation that once one begins to handle the animals, or to train them, copulation ceases and recurs again only after an extended period of rest. The one exception to this finding is the sub-species of D. dorotocephala taken from the spring in Oklahoma that mates regularly and consistently throughout the year even when it is being trained. Planarians are hemaphroditic--that is, during copulation each animal fertilizes the other and each animal later produces a cocoon. When freshly laid, the cocoon of D. dorotocephala is typically an orange-red color; within a few hours, the cocoon turns jet black. Each cocoon contains approximately 16 eggs which will hatch about 14 days after the cocoon is laid. Whenever possible, the cocoon should be removed from the bowl or aquarium in which it is laid if larger animals are present since the adults will typically eat either the cocoon or the young planarians if hungry enough. Once the young worms have hatched, the empty cocoon shell should be left in the bowl with them for several days since the young tend to return to the cocoon for food and shelter. For further information on this subject, see the articles by Marie Jenkins and H. Brown in the bibliography.

A schematic drawing of the nervous system found in the species described above is shown below. While organisms lower on the phylogenetic scale than the planarian do show evidence of having synapses, these more primitive synapses lack the extensive dendritic and axonic ramifications found in planarians and most higher organisms.

Sensory Characteristics

The planarian has specialized receptors for detecting light, chemicals, vibrations and water currents, and various types of pressures. It can also sense temperature gradients, electrical currents, gravity, and perhaps even magnetic currents, although little is known about the receptors which allow the planarian to detect the foregoing stimuli. The common freshwater planarians described above usually have two eye spots which, since they lack lenses, allow the planarians to detect the presence and the direction of light sources, but not shapes or patterns. Specimens of C. foremanii may occasionally

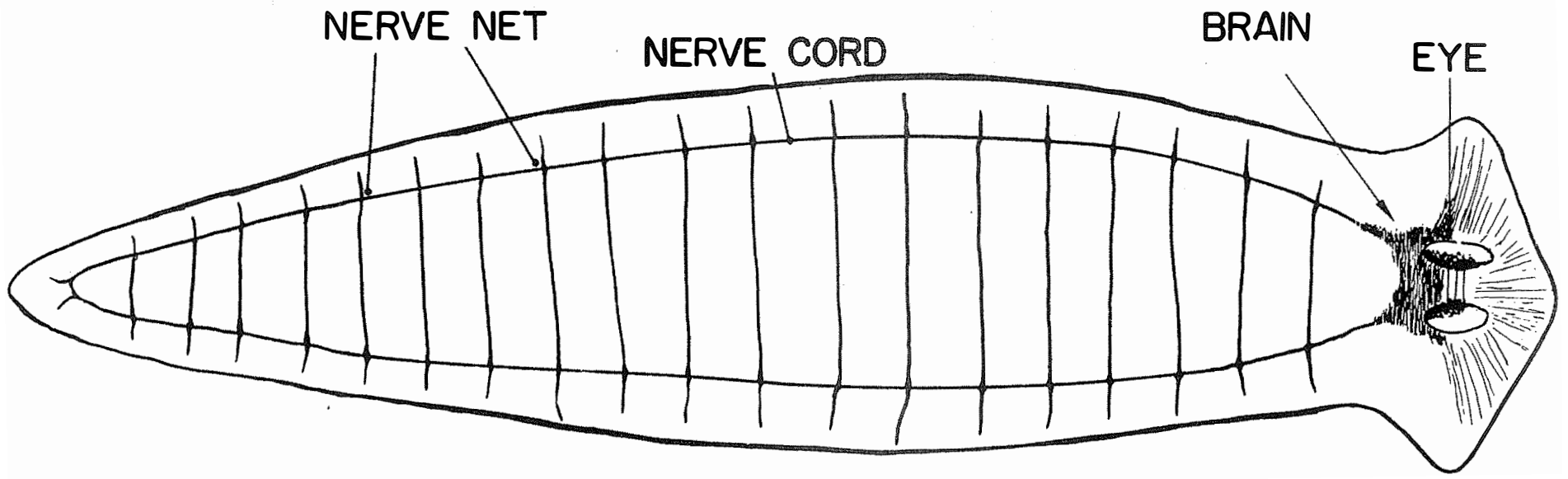


Fig. 5. Schematic drawing of planarian nervous system

be found with three, four, or as many as six distinct eye spots. Planarians are color blind and typically are insensitive to the red end of the visual spectrum. One may often read in the literature that, in addition to the eye spots, planarians contain "light receptors" throughout their bodies. The evidence for such an assumption is questionable at best, and is based on a poorly designed set of experiments done many years ago. Our attempts to replicate these early experiments under more controlled conditions suggest strongly that there is little if any sensitivity to light in the planarian other than in the eye spots. Flatworms are usually "negatively phototaxic," which means that they tend to avoid light. When given a choice, they typically seek out the darkest part of a container. This negative phototaxis may be reversed in some species when the animals are quite hungry, however. When a planarian crosses a sharply defined light/dark boundary, it typically shows a rapid head movement and moves to the darker side. When a planarian has been kept in a dark place for some period of time, and is brought into the light, it typically begins moving and continues to move for a period of several minutes before coming to rest in the light. If one eye spot is destroyed, and the planarian is put in a lighted situation, it will often circle slowly towards its blind side.

The main chemical receptors in the planarian are located in the auricles or "ears". If a piece of food is dropped into water containing a planarian, the animal often moves directly towards the food. Planarians can locate food in their watery environment by following the chemical gradient set up by tiny particles of food as they diffuse through the water. If one of the auricles is removed, and the planarian is put close to a piece of food, it will tend to circle towards the unwounded side. The planarian also seems to have chemo-receptors in the region close to its pharynx, and perhaps on the tip of its pharynx. When the planarian comes close to food, the pharynx often extrudes and moves towards the food even though the body of the planarian is motionless at the time.

Planarians seem to like to keep their underside in contact with some surface and will typically attempt to escape from a situation in which their upper side is in contact with some object. Most planarians cannot swim--they move only when in contact with some surface. Occasionally a planarian may be found crawling upside down on the meniscus (or film on the top of the water). Occasionally they drop from the meniscus to the bottom surface, making slow "righting movements" as they fall. A planarian turned over on its back will show much the same sort of reaction (see photograph). Planarians secrete a mucus as they move (more will be said about this later). Occasionally, as they move across the meniscus, they secrete enough mucus so that when they drop to the bottom, the mucus trails behind them much like a spider web. The planarian then can crawl back up the mucus trail to the meniscus again.

Planarians are attracted by weak vibratory stimuli. This behavior probably has fairly high survival value since many of the small animals on which planarians prey move about by means of the rhythmic beating of legs or cilia. Small animals such as daphnia or brine shrimp often become ensnared in the mucus trails left by planarians--the more vigorously such trapped animals attempt to escape the mucus, the more likely it is that a planarian nearby will be attracted to them. I have seen planarians that were crawling upside down on the meniscus occasionally stop and lower the anterior portion of their bodies when prey such as mosquito larvae were nearby. When a larva bumped into the anterior portion of the planarian, the worm would wrap itself around the larva like a boa constrictor and drop with it to the bottom where it would encase the larva in a web of mucus and devour the larva at its leisure.

X When planarians encounter a weak electrical field, they tend to move away from the anode and towards the cathode. The behavioral ramifications of this sensory characteristic

of planarians will be discussed later.

When given a choice, planarians will pick the temperature best suited to them. While most of the specimens you are likely to encounter can readily be kept at "room temperature," some species such as P. gracilis actually prefer temperatures just above freezing. Hyman states that P. gracilis is killed by temperatures in excess of 30°C. (86° F.). We strive to keep the temperature in our laboratory at approximately 21°C. (70°F.) and have had no trouble keeping P. gracilis alive for long periods of time.

When they encounter a rapidly moving stream of water, most species of planarians tend to move with, rather than against, the current. We will have more to say about this characteristic later on.

For a description of the experiments which have led Brown and his colleagues to the conclusion that planarians can detect weak magnetic fields, I refer you to the articles by Brown listed in section seven.

Distribution.

Planarians can be found in lakes, streams, ponds, and rivers almost anywhere in the United States from mountain tops to tidal basins. They abound in the rivers and lakes close to Ann Arbor. We usually take D. dorotocephala from a rapidly moving stream near the University. In the swifter portions of the stream, the animals are usually found underneath large rocks (we also find a number of leeches under the rocks--be careful!). In the slower, deeper portions of the stream, one can often find the animals moving in large numbers either on the bottom of the stream or on muddy rocks. D. tigrina we usually take from more slowly moving sources such as the Huron River (or from lakes nearby). In such situations, the planarians are typically found under rocks, or crawling under leaves or other debris. Unless one is lucky enough to find a large colony of the animals, one may have to search for hundreds of yards along the shoreline to find even a few specimens. Under such circumstances, one's work is made easier by taking a piece of liver, tying a string to it, and throwing it into the water and leaving it for an hour or more. When one returns, the meat is often covered with planarians. The lazy or less venturesome person may obtain planarians on order from any of the biological supply houses listed below.

Carolina Biological Supply Co.
Burlington, North Carolina, and

Powell Laboratories
Gladstone, Oregon 97027

(see advertisement at end of Manual)

General Biological Supply House, Inc.
8200 South Hoyne Avenue
Chicago 20, Illinois

Misco Corporation
6780 Jackson Road
Ann Arbor, Michigan

Ward's Natural Science Est., Inc.
P. O. Box 1712
Rochester 3, New York

Ward's of California
P. O. Box 1749
Monterey, California

or connecticut valley biologicals

same company

CARE, FEEDING AND HOUSING

The most important thing to remember about taking care of planarians is that they are extremely sensitive to changes in the water in which they live. If the water becomes even slightly contaminated, the worms are likely to die. You must also remember that most tap water contains chlorine which is lethal to the animals. If you obtain your planarians from a nearby stream or pond, it would be better if you also regularly got water from this same source and kept the planarians in the type of water to which they have become accustomed. If you must use tap water, by all means aerate it or let it stand for at least 24 to 48 hours before using it (this allows the chlorine to escape). In our laboratory, we make use of "an artificial medium" made by adding certain chemicals to distilled water. The recipe for making this "artificial medium" follows:

Stock A. Solution:

133.0 Grams NaCl
26.6 Grams CaCl₂
1.0 liter distilled H₂O

Stock B. Solution:

3.8 Grams NaHCO₃
1.0 liter distilled H₂O

Make artificial pond water by adding 10 ml. Stock A Solution and 10 ml. Stock B Solution to each gallon of distilled water.

3.785 liter

We have found it advisable to change the water that the planarians are kept in at least once every week, twice a week if the container is small. At intervals, depending on the size of the container in which the animals are kept, and the type and frequency of food that they are given, the container itself should also be cleaned regularly. The simplest way of cleaning the container is to transfer the planarians to another receptacle and then to scrub the original container thoroughly. The mucus that the planarians secrete sometimes becomes very thick and is not particularly water soluble to begin with--therefore one must wash and wipe the container well to make sure that it is thoroughly clean. If you use soap or detergent in washing out the containers, make sure that you rinse them very thoroughly before putting the planarians back in for even a slight trace of soap can be lethal to the animals.

Housing

Planarians can be kept in a wide variety of containers--in fact, almost anything that will hold water successfully will hold planarians successfully. We store large numbers of animals in long, shallow glass trays like the ones used for baking cakes. A 20¢ glass soup bowl is large enough to house a dozen or more planarians. When we want to keep a single animal by itself (as we do when we are giving it training trials over a period of days), we use glass custard cups. Typically we prefer to keep the animals in glass containers since the glass is inert and not at all harmful to the animals and we can use a grease pencil or laundry marker pen to note on the side of the bowl anything about the planarian that we wish to keep track of. It is not necessary to put a "bubbler" or aerator in with the worms if the container is left open. Planarians can also be kept in a large glass aquarium along with snails and other small aquatic creatures on which the planarians will prey. Since fish have the nasty habit of eating planarians, as do frogs and other am-

phibia, they do not make suitable companions for flatworms. Larger specimens of Dugesia dorotocephala will occasionally attack snails, but snails do not seem to harm the planarians.

Food

Planarians will eat almost anything; unfortunately, they grow and thrive on only a few things. If you want to fatten your worms up in a hurry, by all means feed them fresh liver. According to Hyman, frozen liver (indeed, all frozen meats) are not particularly suitable for feeding to planarians. Our experience is that liver fresh from the slaughter house is superior to frozen liver, but that the animals will live and grow on the latter. Lean beef and other muscle tissue (such as heart) is not particularly suitable. One occasionally reads in the literature that planarians should be kept on egg yolk; Hyman states that both egg yolk and egg white are unsuitable as food. When we first began keeping planarians in the laboratory, we followed the instructions given to us and fed the animals on egg yolk. The planarians readily ate the egg yolk and turned a beautiful yellow color after each meal. Most of the animals grew smaller and smaller rather than larger and larger, just as Hyman said they would. A few of the planarians (the ones that probably couldn't read) did seem to grow and thrive on a diet of egg yolk, however. The difficulty with using liver or some other bloody meat is that the juice rapidly contaminates the water in which the planarians are kept. Thus, if you feed the animals liver, you must remove the residue and change the water within two or three hours after the meat is put in. Because liver contaminates the water so badly, we often feed the animals fresh-hatched brine shrimp instead (for instructions on how to culture brine shrimp, see your local pet store owner who will sell you the eggs at a reasonable price).

If you are going to keep large numbers of planarians on hand for any length of time, you may wish to follow a different technique. Set up a large aquarium (with or without sand on the bottom). Add a few free-floating plants and several large snails. An aquarium of this type can easily house several hundred planarians. For food, set up a culture of daphnia (a small crustacean) in a separate aquarium. The daphnia will thrive on green algae. In our laboratory, we reserve several small aquaria for the growing of green algae to feed to the daphnia which are then fed to the planarians. The algae tanks should contain some free-floating plants, a large number of snails, and guppies or other small fish. The bacteria which form on the snail and fish droppings provide a suitable food source for the algae which will bloom in great profusion if you keep an electric light burning by the tanks. Once the water has turned dark green, transfer some of the water to the tank containing the daphnia (caution: make sure you pour the algae water through a fish net before you put it in with the daphnia; otherwise, a stray fish may get into the tank with the daphnia and devour them all). A thriving culture of daphnia will turn a dark green container of water quite transparent in a matter of a day or so. When all the color is gone, add fresh algae. The daphnia may be caught with a small-mesh fishnet and transferred to the tank containing the planarians. The daphnia will swim about and occasionally get caught in a mucus trail, whereupon they will be readily eaten by the planarians. Once your cultures are thriving, you may actually add so many daphnia to the tank containing the worms that you can culture the daphnia in that tank by adding algae water (poured through a fish net, please!) to the worm tank.

Do not overfeed your worms. A large, healthy planarian can live for six or more months without any food whatsoever. The more you feed your (asexual) planarians, the more likely it is that they will undergo spontaneous fission (or, as we put it, "drop tails"). Overfed worms are sluggish worms. It is probably best to stop feeding the animals several days before you start using them in a psychological experiment and not to feed them

during the course of the entire experiment if at all possible. Planarians kept in the daphnia-rich environment described in the paragraph above are thinner but healthier and much more active than planarians kept in shallow trays and fed liver once or twice a week. If you have sexual specimens and you wish them to mate, however, they will do so more readily when they are fed liver or brine shrimp once or twice a week than when they are kept in a daphnia tank.

Do not overcrowd your animals, particularly if they are cannibalistic by nature. And do not keep cannibal planarians in the same bowl or aquarium with small animals of the same species or even large animals of a different species. For example, if three or four D. dorotocephala are housed with a hundred or more P. gracilis for a period of several months, you are quite likely to end up with two or three dozen D. dorotocephala (fat, happy animals) and no P. gracilis at all!

Miscellaneous Information

As was mentioned above, cocoons should be removed from aquaria housing larger animals as soon as the cocoons are noticed. Whenever an animal undergoes spontaneous fission, the tail section should probably be removed to a separate container, particularly if one is dealing with a cannibalistic species. When large numbers of animals are kept in the same container, it is quite difficult to separate out all the tail drops and this instruction may be ignored. When the animals are housed individually (as during an experiment), tail drops should be removed at once, however. D. tigrina and D. dorotocephala can regenerate a new head within a matter of days. If you haven't taken out the tail drop, you may not be able to tell which is the parent animal and which the offspring.

We prefer to keep our animals on a normal diurnal cycle of light and darkness, since keeping the animals in the dark over-sensitizes them to light. Do not keep your animals in direct sunlight, however--the sun may kill the worms either by raising the water temperature too high or by exposing them to an overdose of ultra-violet irradiation. Try to keep the water temperature to 70° F. or below.

Planarians are subject to a number of diseases and parasites. P. gracilis, for example, may occasionally lose all of its pigmentation and turn almost completely white. If you notice an animal that is changing color, remove it from the aquarium at once and keep it separately. If the animals still in the aquarium continue to show signs of sickness, change the water very often and reduce the number of animals in any one aquarium as drastically as you can. D. tigrina and D. dorotocephala occasionally sprout strange protuberances on their body or develop "holes" (usually just posterior to the head region), such aberrances usually being due to the presence of destructive bacteria in the colony. Once a few of the animals have shown signs of this sort, the entire colony is usually doomed and one might as well order a new supply of animals.

"Tail Drops "

Planarians tend to "drop tails," that is, undergo spontaneous fission whenever they are overfed or roughly handled. The healthier (but thinner) your animals are, and the more gently you treat them, the less likely it is that they will drop tails. The stronger the electric shock that you give them, the more that you punish them, the more trials per day that you give them and the more days per week that you train them, the more likely it is that they will drop tails. Just before a planarian undergoes spontaneous

fission, it becomes hypersensitive to a variety of stimuli and the less normal its behavior becomes. If you are training an animal and it drops a tail, you should discard the tail, and let the head rest for at least 5 or 6 days before testing it again. It would be much better, in fact, if you discarded that animal entirely and did not include its results in your experiment. However, since D. tigrina will often drop a tail if one merely says unkind things to it in annoyance, such discarding may not always be practicable.

Section III

APPARATUS AND PROCEDURE

James V. McConnell

Introduction:

There are two basic laws of human behavior that each person experimenting with lower organisms must take into account if his research is to be well planned and well executed; (1) All humans are anthropocentric; (2) all humans are lazy. It is commonplace in most psychological laboratories that the person who works with white rats for many years comes to think of them as being "people." Rats are not people; neither are planarians. Yet when it comes to deciding what responses a given species can best be taught, or even whether or not a given animal can learn, most experimenters tend to think in terms of human capabilities rather than, let us say, invertebrate capabilities. No one expects a dog or a cat to be able to learn to recite Shakespeare's sonnets or the Star Spangled Banner; yet there are those scientists who, when asked to define "learning," will frown and reply, "well, it's like, you know, it's like you learn a poem." Such definitions tell us precious little about "learning" in organisms other than man (and indeed precious little even about learning in humans). The only way you will ever discover "how much" a given organism can learn is by testing the organism in a variety of experimental situations. If you decide on a priori grounds that planarians are too primitive an organism to be able to learn a maze, it is quite unlikely that you will ever get around to testing them in such an apparatus. A scientist may argue from a variety of theoretical viewpoints that flatworms are too stupid to learn mazes; the planarian, however, never having heard of these fancy scientific theories, goes right ahead and learns a maze! It is quite likely that when we say an organism is "not intelligent enough" to perform a certain task, what we really should say is that we are not intelligent enough to learn how to train the animal adequately. Planarians can learn many tasks, but they do not learn these tasks in exactly the same way that humans learn. If you are to train planarians well, you must begin by observing them in a somewhat natural environment to determine exactly what their behavioral repertory is. Then you must come to "think like a planarian" (that is, you must attempt to see the world as a planarian sees it) if you are to design experimental situations that will really test the animal's capabilities.

One of the "great psychological laws" was first propounded by Zipf, who noticed that most organisms tend to take the shortest possible path to get to food in an open-field situation. Zipf called this tendency "the law of least effort." Humans are probably as subject to this law as are rats or planarians. It is much easier to rush into an experiment and to do it in "a quick and dirty" manner than it is to plan and execute the experiment adequately. In like fashion, it is simpler to adapt to planarians a given psychological technique that is standard for rats or humans than it is to invent a totally new method of training that will take advantage of the planarian's known biological characteristics. An experimenter appears to save more time by giving his planarians hundreds of trials all at once than if he spreads the trials out in tedious fashion, a few each day. The fact that neither rats nor worms learn particularly well when the trials are all bunched together apparently does not deter the usual experimenter from "saving time." Behavioral research is dull; it takes an enormous amount of time and patience to perform such studies well. The experimenter who always obeys Zipf's law will discover too soon that his experimental

subjects seldom obey him!

I. What is learning?

A. Definitions, measures, criteria, and baselines

There are probably as many definitions of "learning" as there are people to define the term. There is, however, some cummunality to most of the definitions. In general, in psychology we speak of "learning" as being a "fairly regular and uniform change in behavior caused by or correlated with some change in the organism's environment (usually under the control of the experimenter). Usually this change in the behavior can be seen (post hoc) to be of an "adaptive" nature, and usually it comes about because we have re-inforced (rewarded) the organism for having changed its way of behaving. Learning is, however, something that takes place inside the organism; learning can never be seen by the experimenter in and of itself--he can only see the change in the organism's behavior. For this reason, psychologists often call learning "an intervening variable." Those changes that we make in the animal's environment in order to force the animal to change its behavior are called "independent variables"--that is, variables over which the experimenter has direct control. The changes in behavior themselves, over which the experimenter has but indirect control, are called "dependent variables" because their occurrence depends on (and is a measure of) the independent and intervening variables. It is very important to remember that learning can never be measured directly--we look at the changes in an organism's behavior and we infer that learning has or has not taken place, but such decisions are always a matter of inference, not of direct observation!

According to some theories, learning is "an increasing tendency of an animal to repeat a response for which it has been reinforced (rewarded)." This type of definition, which works well in some situations but is inappropriate to others, does have the benefit of pointing out one critical feature of the learning situation--the fact that behavioral changes are the end products of both positive and negative factors operating within the organism. Let us assume that we are training a planarian in a simple maze that consists of a starting alley (painted a neutral grey) that connects at right angles with two other alleys, one painted white, the other painted black. As the planarian attempts to escape from the grey starting alley, it comes to a choice point where the three alleys meet and must there decide whether to enter the white or the black alley. Let us further assume that we wish to train it to go to the white alley. Each time the planarian enters the black alley at the choice point, we punish it in some fashion (negative reinforcement). Each time it enters the white alley, we reward it in some fashion (positive reinforcement). At the beginning of training, the planarian tends to enter the white alley as often as the black; after being rewarded time and time again for entering the white alley, and after being punished time and time again for entering the black alley, the planarian tends to enter the white alley significantly more often than it does the black. We now say that the planarian has "learned" to enter the white alley because the response of entering the white alley was positively reinforced and the response of entering the black alley was negatively reinforced. Here learning can be seen to result from at least two different factors: (1) those positive factors which encourage the animal to approach the white, and (2) those negative factors which discourage the animal from entering the black.

In almost all learning situations, one can find both positive (excitatory) factors and negative (inhibitory) factors operating. You must realize that both the positive and the negative tendencies affecting the organism's behavior are learned and that the behavior which the organism displays in the maze is a sort of summation of both types of

factors. There are many situations in which one may attempt to train an animal to approach a given goal (an excitatory tendency) in which the organism does not seem to learn at all because of the presence of overwhelmingly strong inhibitory tendencies. For example, suppose that the white alley in the maze had, unbeknownst to us, a very rough surface that the animal disliked intensely. Under normal situations, the worm would tend to avoid this alley and enter the black alley all the time, but we punish the animal if it enters the black and reward it only if it enters the white. Now the animal is faced with a difficult choice--no matter which alley it enters, it will receive punishment of one kind or another (although we do not realize, as experimenters, that this is the case). Under these circumstances, the planarian is likely to refuse to enter either alley, it may turn over on its back and go into convulsions, it may enter first the white and then the black alley, trial after trial, hunting desperately for the lesser of the two evils; or if the black alley is somehow less punishing than the white (although we, as experimenters, would not see the situation in that light), the planarian may pick the black alley all the time. Most of us would look at this "aberrant" type of behavior as being non-adaptive and perhaps might even cite this as evidence that planarians "cannot learn." What we should say in such circumstances, of course, is that we as experimenters "cannot learn" how to train planarians! It is not easy to analyze a given experimental situation and to determine from such an analysis what the stimulus factors are (that is, what the conditions are in the external world) that affect the worm's behavior, for the stimuli to which a worm responds are not likely to be the same stimuli to which a human would respond. The planarian that comes to enter the white alley more and more often during training has most certainly learned something about the maze, but it may not have learned what you think it has learned nor may it have learned for the reasons you think it should have learned! And the planarian that obstinately refuses to enter the white alley even after hundreds of trials may well have learned a great deal more about the maze than the animal that enters the white alley readily!

As I indicated earlier, learning is typically accompanied by a change in behavior. If we wish to measure learning, then, we must set up an experimental situation in which behavioral changes can take place and be easily measured. So we hunt for a situation in which the animal gives a specific, well-defined response at a particular response rate before training begins; if after we impose a training schedule, the animal gives a different type of response, or responds at a much different rate, we can usually be sure that learning has occurred. In short, in most situations we measure both the qualitative and the quantitative aspects of the animal's behavior both before and after training.

As an example, let us look at "classical conditioning." The noted Russian physiologist, Ivan Pavlov, noticed that his animals salivated whenever he blew food powder into their mouths (he was actually studying the physiology of digestion, not learning). Pavlov also noticed that after he had given the dogs food many, many times, they would begin to salivate as soon as he entered the laboratory without waiting for him actually to give them the food. Pavlov assumed that the dogs did not have to learn to salivate when presented with food--he called this "instinctive behavior." The food powder then was an unconditioned (unlearned) stimulus that innately evoked or elicited an unconditioned (unlearned) response, that of salivation. The unconditioned stimulus is always denoted by the letters UCS; the unconditioned response by the letters UCR. An untrained dog almost never responds by salivating to the stimulation of someone's walking into the laboratory; the stimulus "walking into the laboratory" is therefore "neutral" insofar as the response of salivation is concerned (although, of course, the dog may respond by barking, perking up its ears, etc.). This neutral stimulus Pavlov called the "conditioning stimulus" (or sometimes the "conditioned stimulus") because it seemed to have no innate connection with the UCR of salivation. The conditioning stimulus is abbreviated CS. In Pavlov's first experiments, the CS

(entering the room) always preceded the UCS (food powder to the dog's mouth) by a few seconds; the UCS always elicited or evoked the UCR (salivation). Eventually, as we have seen, the CS came to elicit the UCR even without the presentation of the UCS. At this point, Pavlov decided that the dogs had been "conditioned" to salivate when he entered the room; there was obviously now some sort of connection between the CS and the UCR, so Pavlov decided to describe the latter by the term "conditioned response" (CR).

Now there are several points about Pavlovian conditioning that must be pointed out immediately. To begin with, Pavlov (and most other experimenters) discovered that the CR was seldom exactly the same as the UCR. The dogs did not salivate in quite the same way when Pavlov entered the room as they did when food powder was blown into their mouths. The important thing was that the animals responded to the CS in a way that they had apparently never responded before the training took place. Second, Pavlov discovered from a set of not entirely adequate experiments that the CS "had to" precede the UCS if learning were to take place, and that the closer together in time the CS and the UCS were, the faster and the better the learning. More recent experiments have called into question this particular finding, but we will discuss that later in this chapter. The third point of interest has to do with the "neutrality" of the CS. Planarians are small and it is usually difficult to observe all of their reactions with the naked eye. Most experimenters therefore tend to restrict their conditioning experiments with planarians to situations in which rather gross or large body movements can serve as the UCR. The UCS is typically something like an electric shock passed through the water which causes the planarian to contract or to give a gross bodily movement each time the stimulus is applied. But what should we use as a CS? Suppose we pick a very faint, flashing red light as our CS. We present the weak, red, flashing light for several hundred trials, each time pairing it with an electric shock. The shock always causes the animal to respond, but the light never does even after all the training trials. Can we conclude from this experiment that the planarian could not be conditioned? No, for we must prove first that the worm actually could detect the CS in the first place. If we were unfortunate enough to choose a weak, red, flashing light, we would never be able to condition worms to respond to it since planarians are insensitive to visual stimulation in the red end of the spectrum!

How can we be sure that the planarian does sense the CS? Why, only if it occasionally responds to the CS, and since the only response we bother to notice (typically) is a gross bodily reaction, we have now set up a situation in which the CS is actually a weak UCS! Here we do not hope to achieve conditioning by pairing a "neutral" CS with a UCS, but rather we hope to change the rate of response that the planarian gives to a weak UCS (the electric light) by pairing this with a strong UCS (electric shock) to which the organism always responds. If one carries this analysis a little further, one soon discovers that all CSs are in fact weak UCSs, but that perhaps is another story.

* Whenever one undertakes a conditioning study with planarians, then, one must begin by presenting the so-called CS a great many times in order to determine what the naive (pretraining) response rate to the CS is. It goes without saying that one must adjust the intensity or the type of the CS properly before conditioning actually is begun; if the naive response rate is too low, one cannot be certain that the planarian actually perceives the stimulus, and if it is too high one can never be certain that conditioning has actually taken place. We have found over the years that a naive response rate to the CS of approximately 10-20% is optimal. One can usually adjust the response rate to a CS such as electric light by moving the bulb itself closer to or further away from the conditioning trough, or by increasing or decreasing the length of time that the light is on prior to the onset of the shock (UCS). See below for further details.

By now you will have realized that all definitions of learning are quite arbitrary. It goes without saying that the point during training at which one is willing to state that an animal "has really learned" is also an arbitrary decision that each experimenter must make for himself. If we gave you a long poem to learn "by heart," and you studied it for quite some time, how would we be sure that you "really knew the poem?" We might state arbitrarily that whenever you could repeat the poem once perfectly, without prompts, that you had "learned." However, as all students and teachers know, material which is learned "perfectly" to this criterion on a given Tuesday cannot always be repeated perfectly two weeks later if the student has not practiced inbetween times. Furthermore, suppose we made the poem so long and so difficult that you could never repeat back more than half of it without prompting. Would this mean that you were incapable of learning anything about the poem? Or suppose that in addition to repeating back the poem, we also required you to stand on your head and play "The Jolly Farmer" on a piano with your toes. Perhaps, under the best of circumstances, you could learn the poem rather well, but the additional requirements might make it so difficult to repeat the poem, and so annoying to you, that you simply wouldn't bother putting forth your "best effort" under the circumstances. Should we then conclude that you were stupid, or that the experimenter was?

Psychologists are usually willing to state that an organism has "learned" a given task if its rate of responding to the CS increases significantly because of the training given the organism. But what do we mean by "significantly?" This term has a rather precise mathematical meaning far too complicated for us to discuss in this particular Manual; in essence, what we mean is that the change in the rate of responding is great enough so that we are reasonably convinced that the change was not due to some chance or random factor other than the training itself. In most conditioning experiments, we have a choice of at least two different ways of proceeding. Suppose we are using light as our CS and electric shock as our UCS, and that the animal's naive (pretraining) response rate to the light is 20%. We can decide ahead of time that our "criterion of conditioning" will be 90%-- that is, we will continue to pair the CS and the UCS until the animal has shifted from a 20% response rate to a 90% response rate, at which time we will be satisfied that the animal has "learned." This is a very fine procedure provided that all the animals are capable of reaching the 90% response rate criterion under the circumstances. But suppose that we find that only half of the animals reach this criterion even after we have given them several hundred trials. Does this prove that half of the animals have learned while the other half have not? Suppose, for example, that half of the animals reached the 90% response rate, but the other half reached only 89%--are the latter animals really "too stupid to learn?" A more logical way of setting a criterion of conditioning is the following: one gives all of the animals a set number of trials. At the end of this period of training, one calculates the average or mean response rate for the last few trials for all of the animals and compares it with the initial response rate. Suppose in this case that the terminal response rate for all of the animals was, on the average, 50%. Could we say that planarians had learned? Most psychologists would be willing to say "yes" provided that we showed mathematically that this increase in response rate were statistically significant (using a mathematical formula too complicated to describe in this manual). Either of these definitions of learning is acceptable, and we have used both in our laboratory from time to time. Which method you choose to employ depends on what it is about planarian behavior you are attempting to study. The important thing to remember is that both types of definition are entirely arbitrary.

B. Controls and Control Groups

There is probably no other group of scientists as enamored of the use of "control

groups" as are psychologists; indeed the entire history of scientific psychology may be viewed as a continuing search for better controls. All well and good, you say, but what does a psychologist mean when he speaks of a "control group"? Well, let us suppose that you believe you have discovered a new drug which will cure headaches. How would you set about proving to the U.S. Food and Drug Administration that your pill really does what you say it does? Obviously, you would have to run an experiment. Suppose you took 100 people who had headaches and gave them the pill, and then several hours later you asked these 100 people whether or not they still had headaches. Probably 90% or more would say that the headache no longer bothered them. Could you conclude from this type of experiment that your pill had "cured" these 90%? The answer is, of course, no. Most headaches do not last for more than a few hours whether the person takes a pill or not. The FDA would require that you found another 100 people (matched as closely as possible to the original 100) who also had headaches and that you not give this group of people the pill. At the end of the same length of time, you would ask the second group whether they now had lost their headaches. Suppose only 30% of this group said that their headaches had now disappeared. Would this mean that your pill cured 90% minus 30% or 60% of the people? After all, the second group of 100 people served as a control group to measure what would have happened had the original 100 not been given your pill. Unfortunately, in this case, one control group is not enough. Experiment after experiment has shown that people with headaches tend to get well if they are given any kind of medication whatsoever, even if it is only a pill made of sugar! In this case you would obviously need a third group of 100 individuals with headaches who are given a "sugar pill" (the technical name is placebo). If the recovery rate is, say, only 50% in the placebo control group, then and only then can you be fairly certain that your pill has some medicinal properties. As we will mention below, there are still further controls you would wish to institute, but their description can wait for a few minutes.

Let us return to classical (Pavlovian) conditioning. Suppose we are using light as our CS, shock as our UCS, and we pair the light and the shock for 200 trials. If the naive response rate to the light was 20%, and after training the response rate to the light was 90%, can we say that the animal has actually been conditioned? The answer is "no," not without the use of some control groups. Perhaps just giving the animal several hundred trials of light somehow "sensitizes" the animal so that it now responds to the light much more than it did at the beginning. How can we control for such "sensitization?" The simplest way is by having a control group of animals that receives trial after trial of light but which are never given the UCS (shock). In such cases (particularly with planarians), one typically finds that the animals respond less and less to the light. For example, in 1955 Thompson and McConnell reported an initial response rate to light of 28% in their planarians. Animals that were given 150 trials of light paired with shock had a terminal response rate to the light of almost 50% (a statistically significant increase). A "light control group" was given 150 trials of light only; their naive response rate to the light was again approximately 28%, but at the end of 150 trials they were responding to the light less than 10% of the time. This decrease in responsivity to the light is known technically as "habituation," and its occurrence suggests strongly that the increase in response rate found in the experimental animals was not due simply to giving these animals 150 trials of light. But what about the shock? Perhaps shocking the animals 150 times "sensitizes" them to the onset of the light. To control for this, Thompson and McConnell had another control group that was given 150 trials of shock only (never paired with light). In this control group, the animals were given 5 trials of shock, followed by one "test trial" of light only, followed by another five trials of shock, etc. The light and the shock were, of course, never paired (that is, light and shock were always presented at least 20 seconds apart). In the case of the "shock control animals," the naive response rate was around 30%, but had dropped to less than 25% at the end of the 150 trials. This "habituation" to the shock also suggested

that the increase in response rate found in the experimental animals was not due to some form of "sensitization." The design of this latter control group could have been improved had it been given an equal number of shock-only and light-only trials (that is, 150 of each), in which case it would have become a "pseudo-conditioning control group."

Pavlov's experiments led him to theorize that the CS had to precede the UCS by a very brief period of time or "true" learning would not take place. Pavlov, you see, believed that the CS came to act as a "signal" that the UCS was soon to appear, and that the animal came to associate the CS and the UCS because they always appeared so closely together in time. This theoretical framework led Pavlov to believe that if one merely presented the CS then removed it and then several seconds later presented the UCS, learning should not occur. Unfortunately, not all animals have apparently bothered to read Pavlov's theory, and many of them show an increase response rate even when the CS and the UCS are separated in time by several seconds. Since, according to the theory, this could not be "true" conditioning, it was called "pseudo-conditioning" and has typically been ignored ever since except as a type of situation one wishes to avoid. Whether or not "pseudo-conditioning" is really a form of learning is rather a knotty problem best not discussed in these pages.

Control groups serve to reassure the experimenter that the changes he observes in an organism's behavior are the result of the specific experimental manipulations that he has made, and not due to some chance or random factors or to the mere fact that one has done something to the organism rather than merely leaving it alone. The importance of using control groups cannot be overestimated, and the clever experimenter is the one who almost intuitively knows which control groups are necessary in any given situation. In general, of course, one chooses control groups to rule out every possible logical explanation for a change in an organism's behavior other than the explanation one believes is really true.

C. Other Types of Controls

Let us return now to the "pill" experiment described above. Even if we use a control group which is given no pill at all, and another control group which is given a placebo, can we be absolutely certain that the greater recovery rate found in the experimental group was due to the chemical ingredients in the medication they received? The answer is, unfortunately, a resounding "no." Human beings, like most animals, are quite sensitive to a variety of environmental stimuli that most scientists never bother to measure. In the past, a great many pharmacological studies have yielded inaccurate results for a very odd reason. Suppose you were the person who had discovered this new medication for headaches, and suppose too that you were in charge of the experiment to test its efficacy. Obviously, you have a great deal of pride and prestige (not to mention financial gain) riding on the outcome of the experiment. You want the pill to work, and so do your associates in the experiment. Under these circumstances, don't you think that you might very well (quite unintentionally) handle the placebo control group and the experimental group in slightly different fashion? You might well think that you were treating the groups identically, but study after study has shown that under these circumstances, the experimenter often biases his results by subtly suggesting to the experimental group that their pill really works, while equally subtly suggesting to the placebo control group that their pill doesn't!

Let us repeat--the experimenter usually is completely unaware that he is exercising this subtle influence.

It was to control for this type of bias that a procedure now known as the "double-blind" technique was devised, which would operate in the "headache experiment" as follows: one team of experimenters would prepare an equal number of placebo pills and real pills, and would make sure that both types of pills appeared identical in every way possible. Each type of pill would then be given a code number, but only the first team of experimenters would know which code number stood for which type of pill. A second team of experimenters (which didn't know the code) would be in charge of passing out the medication to 200 people having headaches. Since there were 100 placebos and 100 real pills, half of the 200 people would be the experimental group and half would be the control group, but neither the experimental team that passed out the pills nor the subjects themselves would have any idea until after the experiment was over who was in which group. Once the final reports from the subjects as to the effects of the pills had been gathered, then and only then would the code be broken and would both teams of experimenters find out which subject had received which pill.

In running planarian experiments, or experiments with any other animal, it is just as important that one control for "experimenter bias" as it is when running drug studies. It is quite possible to bias one's observations of an animal's behavior quite unconsciously if one knows ahead of time whether the animal is in an experimental or in a control group (and hence knows what sort of response rate the animal is supposed to show if one's theory is correct).

There are several types of methods for eliminating "experimenter bias" in planarian studies. To begin with, it is important to be as objective as possible in recording the animal's responses. Did the worm really wiggle, or was that just a figment of your imagination? If two or more people are watching the same worm at the same time, and recording its responses independently, you have a good measure of control over the reliability of the observer. In our laboratory, we typically find that trained observers agree almost 100% of the time when observing the same animal. The important word in that last sentence, by the way, is trained. If at all possible, when you first begin to experiment with planarians, you should serve as an apprentice to someone who has already learned the techniques for handling the animals and for scoring their responses. If such a procedure is not possible, try to talk one of your friends into observing the animals with you so that you can be sure that someone else sees the same sorts of reactions that you see.

Another type of control should be used in the following situation. Suppose you were giving your animals 25 trials a day and are running them until they reach a response rate of 90%. This sort of learning typically takes several hundred trials to achieve, so you will be running the animals over a period of many days. Now, off hand, you would expect that an animal that has been given 125 trials would respond at a higher rate than an animal that had never been given any training trails at all; and since you would expect the former animal to respond more than the latter animal, you might well see what you expected to see. Having a second observer does not help much in this case, since he might be expecting the same sort of results. To control for this type of bias, it is usually necessary to arrange things so that the person actually running the animals has no knowledge from one day to the next of which animal has had previous training of any kind. This means that the experimental animals (and the controls) should be given code numbers which are changed from one day to the next by someone other than the person who is going to be training the animals. Furthermore, if you start running all of the experimental animals at the same time, so that on any given day they will all have had the same amount of prior training, it is usually wise to throw in "ringers"--that is, each day to add one or two animals that have never received any prior training whatsoever. Since the person actually running the

animals knows that he will be training both naive and experienced (sophisticated) animals on any given day, he will not expect any particular animal to have either a high or a low response rate. Not knowing what to expect, the experimenter cannot easily bias his results under such circumstances. And obviously, if you were performing some of the "cannibalism" or "chemical injection" studies described below, there are rather special control groups that you will wish to employ. We will discuss these particular groups at a later time.

It may well seem to you that the use of control groups is a great waste of time and that the controls for experimenter bias are something you can easily do away with in your own experimentation. If you believe this strongly, let me recommend that you get out of science immediately and go into art or the humanities instead. No scientific experiment is any better than the control groups it includes. I would estimate that better than 50% of the experiments performed in the general field of psychobiology in the past (including studies by the greatest names in the field) are probably invalid (or at least open to serious question) because the experimenters did not perform adequate controls, particularly the controls for "experimenter bias." There are enough bad studies in the literature as is. Anyone who wishes to increase this terrible mass of useless experimentation by adding his own inadequately controlled studies is merely asking for trouble.

One final point. At any given moment in the history of science, it is likely that no individual knows enough about the subject he is working with to be able to design the "perfect" experiment. Time and time again, particularly in the study of behavior, the discovery of new information about a given organism or a given experimental situation has called into question the results of past experiments which seemed "classic" when first performed. The wise scientist is absolutely certain about absolutely nothing, and continually re-evaluates yesterday's "known facts" in light of today's "new evidence."

II. Variability

A. If you read any report of a learning study which appears in a "professional" scientific journal, you are likely to be impressed by two things--the relative ease with which the experiment was performed and the relative smoothness of the curve which describes the rate at which the animals "learned." However, anyone who has performed many such studies himself will not be so taken in; few experiments really go "smoothly" in anybody's laboratory, and the "regularity" with which most animals seem to acquire a new habit is a function more of the way the data are reported in the journal than it is of the way the animals actually learned. Nonetheless, one of the standard myths in the field of learning is that one encounters but little day-to-day variability in one's experimental animals. In fact, worms, rats, dogs and cats are just as different from one day to the next as human beings are, if not more so. Worms have their good days and bad, their ups and downs, just as do students and teachers. Perhaps the only difference is that worms are under no social constraints "to behave properly"--if they do not feel like "performing" on any given day, they simply don't perform. Variability in an animal's behavior is a fact of life that each experimenter must learn to face. The question that should concern us at this point is this--what causes this variability?

Most psychologists assume that an animal's performance at any given instant in time is the direct result of both external and internal factors which act in combination to shape the organism's behavior. Anything in the external world around the animal (the temperature of the water, the acidity of the water, the shape of the apparatus it is in, the amount of light falling on the animal, etc.) can theoretically have an influence on the

organism's behavior. In most learning experiments, we attempt to hold all of these external stimuli constant except for the one or two (the CS and the UCS) that we are manipulating. In general, the more successful we are in holding these extraneous stimuli constant, the less variable the animal will be from day to day in its behavior. However, it is terribly difficult for even the most astute psychologist to know precisely what external stimuli have the greatest effect on an organism's behavior and we often fail even to identify what all of the important external stimuli are! And while the psychologist can hope to have at least some minimum control over the external environment, the conditions inside the animal that likewise affect its behavior at any given moment in time are often far beyond the psychologist's control. Does hunger affect the animal's speed of learning? We believe it does, so we attempt to make sure that the experimental subjects are fed on a regular schedule. Does the age or the size of the animal somehow affect its responsivity to various external stimuli? We believe so, therefore we attempt to make sure that all our animals are approximately the same size and of the same age. Does a worm that is "not feeling very well" perform as well as a planarian that is in good physical health? Since we believe the animal's physical condition is an important variable, we handle the animals extremely gently in order not to damage them, and if any animal seems to have become ill through no known fault of ours, we put it on "sick call" for a few days until it has recovered. Since we are confident that past experience influences the animal's present behavior, we attempt to make sure that all of our experimental subjects have had pretty much the same experiential background before the experiment starts; typically this means that we run only "naive subjects" (that is, animals that have had no prior experimental training). But notice, please, that the only control we have over the animal's internal environment comes from manipulations that we make or have made in the past in its external environment.

B. Planarians have a very small but relatively complicated brain; humans have an extremely large and frighteningly complicated central nervous system. What behavioral differences can we expect to obtain between humans and worms as a direct result of their neurophysiological differences? Obviously humans can perform many tasks and learn many things that planarians cannot. But the size of the human brain gives us one further advantage over the flatworm--we can surely learn to ignore fluctuations in our environment to a much greater extent than can the simple flatworm. The larger an organism's brain is, the more its behavior is under the control of its own central nervous system; since planarians have a very small brain, we would expect them to be much more sensitive to minute changes in the external environment than would men be. It is apparent then that we must exercise much greater control over the stimulus world that we expose our planarians to than we would if we were attempting to train mere human beings.

C. Learning is usually thought of as being the end product of positive, excitatory tendencies and negative, inhibitory tendencies which build up within the animal. We know a great deal more about the conditions that promote the creation of positive, excitatory tendencies than we do about those conditions which promote or facilitate the build-up of inhibitory tendencies within the animal. Much of the day-to-day changeability in an organism's behavior is probably due to the relative amounts of positive and negative tendencies operating within the animal at that moment. Most experimental psychologists attempt to minimize the negative tendencies as best they can so that the behavior they observe is chiefly a function of the positive tendencies created by the learning experiment itself. In the past, we have discovered that planarians, like people, grow tired if one forces them to run too many trials in a short period of time, or if one gives them too much negative reinforcement in a short period of time. Planarians that are given a great many trials in one day, and are given training every day, will surely show much

greater variability than animals that are given but a few trials per day and are given frequent "days off" to rest. Many of the negative factors inside an organism will dissipate or diminish if the animal is given frequent rest periods; the excitatory tendencies in general tend not to dissipate as rapidly. The wise experimenter will always resist the tendency "to get it over with quickly" by over-training his animals since he will realize that such procedures can be depended on to make the animals quite undependable.

D. Variability is a biological fact of life which all experimenters must come to live with. But the more you learn about the external and internal factors which influence your planarians' behavior, the more rigor you employ in the design and execution of your experiments, and the more gently you handle your animals, the less the variability will be.

III. The Response

A. Types of responses

As we noted above, planarians are rather small and only large or gross movements of the body are typically visible to the naked eye. In the case of maze learning, the size of the response is unimportant--it matters little whether the animal creeps, dashes, or corkscrews into the correct arm of the maze so long as it eventually gets there. In the case of classical (Pavlovian) conditioning, however, the type or "shape" of the response is of critical interest. Planarians have two major types of movement--a smooth or gliding motion (thought to be accomplished by the rhythmic movement of the cilia on the animal's ventral or bottom surface) and a "humping" or "inching" movement which is usually referred to as "ditaxic" or "amoeboid" movement and which is accomplished by muscular contractions. The latter type of movement is observed chiefly when the planarian has been disturbed by something in the environment. Planarians also show such minor movements as a brief lifting of the head, a sudden contraction of its anterior or posterior end, a twitching of its auricles, etc. (not to mention the rather specialized movements involved in eating and reproductive activities). When badly stressed, planarians often roll over on their sides and show a rhythmic convulsive activity. Any of these responses could be counted as "conditioned responses" under the proper circumstances; the question is, which types of reactions should we record?

In our discussion of classical conditioning above, we pointed out that while the conditioned reaction is not always identical to the unconditioned response, usually the two responses have certain features in common. Therefore, in classical conditioning, we typically record only those responses elicited by the CS which are somehow reminiscent of the UCR. Electric shock, a typical UCS, evokes a strong contraction of the planarian's body if the animal is moving directly towards one or another of the electrodes, or a combined contraction and turning movement if the animal is oriented at some angle across the field of maximal current flow between the two electrodes. Typically, in the latter condition, the animal's head is forced automatically to move towards the cathode or away from the anode. If we are using the onset of light as our CS, we record any major contraction or turning movement the animal makes before the onset of the UCS. In my own laboratory, we usually do not begin a conditioning trial until the animal is gliding smoothly along the bottom of the conditioning trough and is oriented towards one or another of the electrodes. The CS (light) is turned on for 2-4 seconds before the UCS (shock) is turned on, and any noticeable response which occurs prior to the onset of the UCS is recorded. Since the animal is gliding smoothly, we arbitrarily score as a response any noticeable deviation in its direction of movement (i. e. if the animal turns at least 60 degrees away from the

electrode it was approaching) or if it stops or contracts.

As one might expect, different species of planarians characteristically respond in different ways. D. dorotocephala is a much more "nervous" and active animal than is D. tigrina, hence the former is more useful for maze studies than is the latter, although both may readily be used for conditioning experiments. In the case of classical conditioning, however, D. dorotocephala will have a much higher naive (pre-training) response rate than will D. tigrina. Some species of planarians react to the onset of new stimulation with a violent lifting of the entire anterior portion of the animal's body (the "rearing" response). In these species, of course, this type of reaction is usually scored.

Very recent conditioning studies suggest that the head turning response is a much poorer index of learning than is the contraction. Animals run in "clean" conditioning troughs for example, appear to show an immediate increase in head-turns but not in contractions. In like fashion, the differences between experimental and pseudo-conditioning control animals are often maximized if one ignores head-turns and scores only contractions. It may well be the case that when the planarian becomes sensitized to its environment, it responds by making a greater number of head movements, but when it is actually learning, the conditioned response it acquires is that of a bodily contraction. In one sense, this is good news indeed, since the beginning experimenter usually can score contractions much more reliably than he can head turnings. Until the issue is fully resolved, however, the wise investigator will probably wish to record both types of response and report the data on them separately.

B. Scoring the response

Often one wishes to record not only the presence or absence of a CR, but also the type of CR which occurred and its strength. We have found the following scoring system to be of value.

Strength of response: 0--a "goose egg" indicates that the animal gave no visible response on this particular trial. 1--a doubtful reaction at best. A very weak response. 2--a strong, vigorous, marked reaction of one kind or another. When you are adding up the reactions of the animal to determine whether or not it has reached criterion, you may wish to include the category "1" responses either as zeros or twos--that is to force your data into a "response--no response" dichotomy. If you are of a cautious or conservative frame of mind, you will probably wish to include the ones with the zeros; as you become more confident of your powers of observations, you will probably wish to include the ones with the twos. When two observers watch the same animal and score its responses independently, it is (as might be expected) the ones that give the most difficulty.

Type of Response. Turning reactions are typically recorded in terms of the direction in which the animal turned--that is, we record an L for a left turn and an R for a right turn. A contraction of the animal's body we score as a C. A stop is scored as an S, while a vigorous lifting of the animal's head may be scored as an A. If the animal makes two or more responses within the period of time that the CS is on (prior to the onset of the UCS), both responses are noted; thus LC means that the animal first turned left and then contracted. The numeral denoting the strength of the animal's response follows the letters denoting the type of response: thus SA2 means that the animal first stopped then raised its head in a very vigorous and marked fashion.

In classical conditioning experiments, one always makes sure that the strength of the UCS is great enough always to evoke the UCR. Therefore, there is little need to record either the type or the strength of the UCR since it varies little from one trial to another. However, if on any given trial the UCS does not evoke the UCR, you should make note of this fact (and increase the strength of the UCS before running another trial).

It has been my experience that at the beginning of classical conditioning, the planarian will make very few responses to the CS and these will often be type 1 reactions. As the animal approaches criterion, however, type 2 reactions begin to predominate. It is also interesting that many planarians show a combined turning and contraction as their predominant reaction to the CS as they approach criterion--in many instances, indeed, the first appearance of this type of reaction means that the animal will soon reach criterion.

IV. Apparatus.

A. Classical conditioning

The best type of apparatus for obtaining classical conditioning in planarians is a long, shallow trough either semi-circular or V-shaped in cross section. The trough is usually gouged out of plastic or some other inert material and is usually colorless or white in order to make the animals more visible. A simple trough can be constructed from a long plastic tube which is split from one end to the other and then blocked at either end so that it will hold water. A simple trough can also be gouged out of parafin or wax. Special care must be taken to make sure that the surface of the trough is as smooth and as clean as possible since any irregularity is likely to cause the planarian to react. Electrodes should be mounted at either end of the trough and should be of some noble metal (gold, silver, or platinum) if at all possible since metals such as brass will typically release ions into the water when direct current is passed through them. A view of the type of conditioning trough we used for many years is shown in Figure 6.

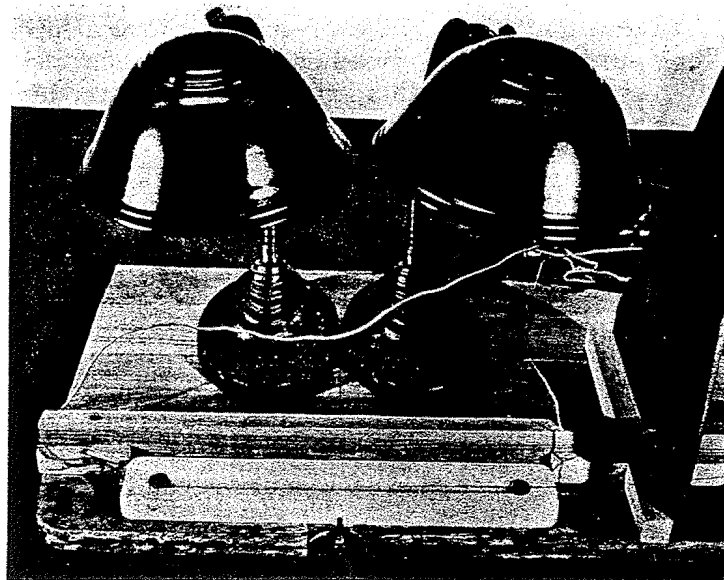


Fig. 6. A pre-1964 conditioning trough for planarians

The trough is long and narrow in order to insure that the density of the electrical current flowing between the two electrodes will be relatively the same no matter where in the apparatus the animal is. If the electrodes are not at the far ends of the trough, of course, the planarian will not be shocked in the same way (if it is shocked at all) if it is behind one of the electrodes as it will if it is between the two electrodes. One can make a very crude but unsatisfactory conditioning apparatus merely by sticking two electric wires in a round dish filled with water; since the current density is quite different from one part of this dish to another, one cannot always be sure that the UCR will be elicited on any given trial, however, and I do not recommend the use of this type of apparatus.

Planarians, like humans, are not only lazy but also tire rather easily. When you first put a planarian into a conditioning trough, it will move about rather vigorously for a minute or two, then usually settle down into a smooth gliding motion as it explores its new environment. However, particularly after it has received a few electric shocks, the planarian has a tendency to cease movement altogether and to come to a halt, often clinging to the bottom of the trough tenaciously. After the animal has been given a minute or so to rest, it can be prompted to movement again by touching it very gently on the tail with a camel's hair brush, or by agitating the water in the animal's vicinity. If a brush is used, one should make certain to stimulate the animal on the tail rather than on the head, since the latter type of stimulation typically causes the animal to cling even more tightly and makes it even more reluctant to move than before the stimulation occurred. We found the type of trough shown above unsatisfactory for two reasons. First, since the animal must be moving slowly in a straight line and oriented towards one of the electrodes before a trial can be started, one obviously cannot begin a trial when the animal is close upon either electrode, for as it reaches the electrode it will be forced to turn around and come back (a condition which might disguise the appearance of any CR). Planarians apparently have no difficulty in learning that they will not be shocked when they are actually resting on one of the electrodes and a sophisticated animal will often make straight for one of the electrodes as soon as it is put in the trough and will show a great reluctance to leave the electrode. Some animals also learn (apparently) that no trial will be started if they are showing either violently agitated movement or if they are not moving at all. It is not at all unusual to find a planarian gliding smoothly and calmly about its home bowl before a day's training begins and then, when one puts the animal in the conditioning trough, to find that it will either refuse to move at all or that it shows agitated movement for a long period of time. Needless to say, when the animal is returned to its home bowl, it resumes its smooth and unexcited gliding once more. Unfortunately, this type of "learning" is seldom recorded or mentioned in the scientific literature.

In order to overcome some of the difficulties mentioned above, we have recently instituted a new type of conditioning trough, shown in Figure 7. Since this new trough is "continuous," and since the electrodes are counter-sunk into each of the four corners so that they are flush with the bottom surface of the trough, the planarian cannot find "an end" to the trough to which to cling. Although trials are not run when the animal is approaching one of the corners, we have so far not noticed that the animals tend to remain in any one part of the trough. It might be thought that a circular trough would be even more effective than a square trough, but a moment's consideration will convince you that the current density is better controlled in a square than in a circular piece of apparatus. In the case of the square trough, the electrodes at opposite corners of the trough (that is, diagonally opposite to each other) are connected together to form one single, common electrode. One set of these diagonal electrodes leads to the anode of the power source while the other leads to the cathode; thus, no matter where in the trough the planarian is, it is always between an anode and a cathode.

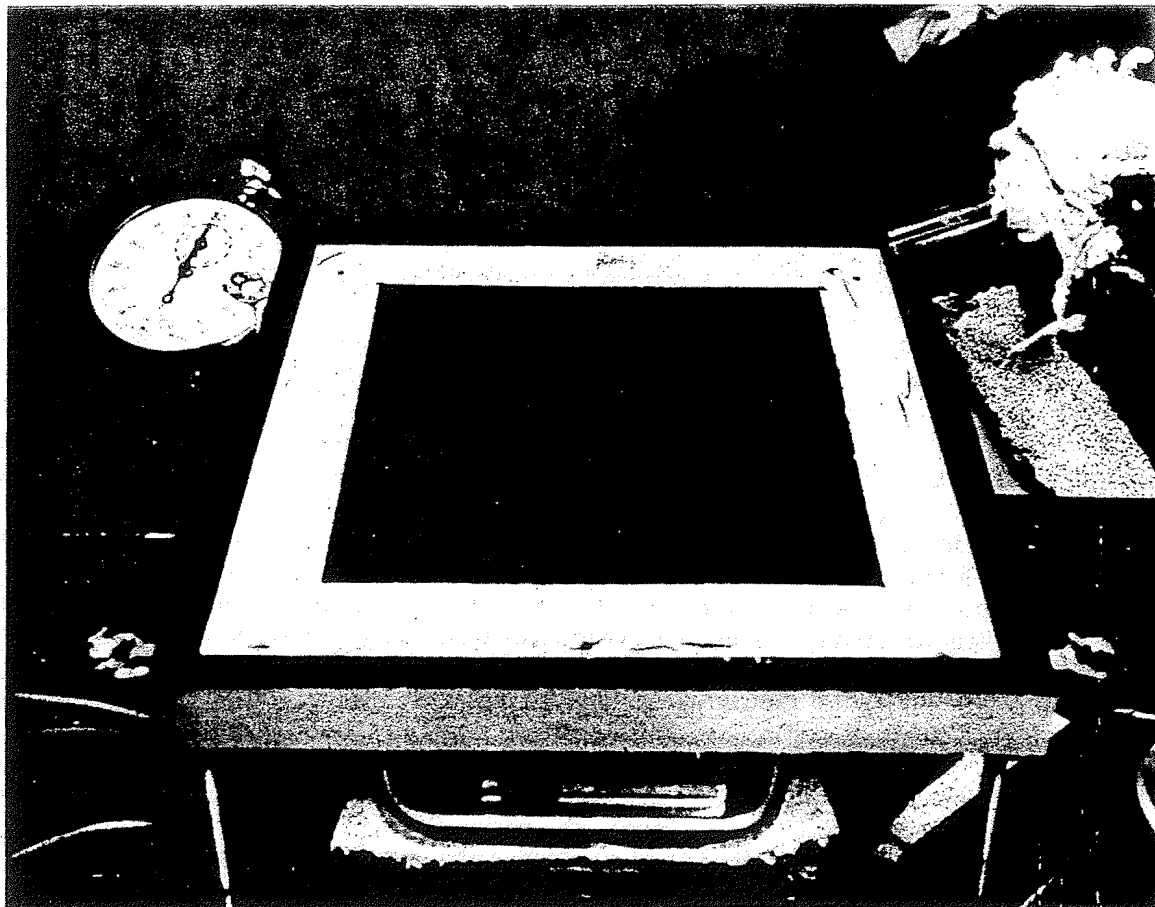


Fig. 7. A "square" conditioning trough for planarians.

If you will look again at the photograph of the square trough, you will notice in the upper right hand corner the small glass tube projecting over the surface of the trough and lying almost parallel to the surface of the water. A weak jet of air from this glass tube passes over the surface of the water in one arm of the trough and sets the water into continuous motion. Planarians have a strong, innate tendency to move with the flow of the water in such situations. If a planarian is run in a square trough without the water's being in circulation, it will usually come to rest in a matter of ten to fifteen minutes; if the air jet is turned on, the planarian will often move for one to two hours before it comes to rest. This modification of the square trough was first introduced in our laboratory by Leroy Vernon.

As of the spring of 1965, there is still considerable argument about the type of shock best suited as the UCS in conditioning studies. Alternating current has been used by only a few experimenters and, in my own experience, is not as effective (and usually more deleterious) than is direct current. If one uses a direct current, it is better to find a means of rapidly pulsing the current flow if at all possible, since a weak pulsed current will usually evoke as vigorous a contraction as will a strong unpulsed current. The use of an Harvard inductorium or an electronic stimulator which delivers a regular square- or sinusoidally-shaped wave is highly recommended whenever possible. If the electrodes in the trough are hooked directly to some non-fluctuating source such as a battery, a 6 volt source should be sufficient. No matter what the source of the current, one must make every effort to arrange the apparatus so that one can control the polarity of the shock. If a planarian is headed towards the anode when the shock is applied, its head will contract first; if headed towards the cathode, its tail will contract first. Barnes and Katzung (see bibliography) have reported that if they always delivered the UCS when their animals were headed towards the anode, no learning occurred; learning did occur

readily, however, when they always shocked the planarians when headed towards the cathode. As we will discuss below, the opposite seems to be the case when maze learning is considered. Although the Barnes and Katzung results have been repeated by Roy John (personal communication), in my own laboratory we have usually obtained the opposite results--that is, anodal shock leads to faster conditioning than does cathodal. The wise experimenter will probably undertake both types of training paradigms at the beginning of his work in order to determine which type of shock seems to work best for him.

The intensity of the shock used in any situation should be just great enough always to evoke a vigorous UCR from the planarian. The exact amount of shock will, of course, vary from one piece of apparatus to another--which is why one should always let the planarian itself determine how much shock is necessary. Begin with an intensity of current too weak to evoke a reaction and, trial after trial, increase the intensity until a level of stimulation is found that always evokes a reaction. This strength of shock can then usually be maintained day after day, from one planarian to another, with excellent results. In this case, oddly enough, it is less useful to know the exact physical dimensions of the shock used in an experiment than the exact behavioral dimensions (UCR) that the shock (UCS) elicits from the planarians.

If one picks electric light as the CS, the first problem one must solve is the exact intensity of the light to be used. In general, the more intense the CS, the higher the naive response rate will be. We find it of some benefit to adjust the intensity of the light so that our naive response rate is no greater than 20%, and to give each planarian at least 50 trials of "light only" prior to initiating light/shock training. Even under the best circumstances, a small percentage of naive planarians can be expected to respond at a very high rate when first given trials of light alone. If after the 50 or so "light only" trials, these animals are still responding at a very high rate, they should probably be discarded (and some note of this be made in the write-up of the experiment). Obviously, if the percentage of these animals is much greater than, say 10%, the intensity of the CS itself should be sharply reduced. If a large number of the animals still respond at a very high naive rate, it is probable that some other factor (such as illness of the animals) is responsible. Whatever the intensity of the CS used is, one should make sure that the light is as uniformly distributed over the entire length of the conditioning trough as possible. Planarians are quite sensitive to minute variations in both the intensity and in the directionality of a light source and may therefore respond more often in one part of the trough than in another if the distribution of the light is not relatively uniform.

The intensity and the uniformity of the background illumination is as important to control as that of the CS. Obviously, planarians cannot be run in complete darkness between trials since one could then never determine whether or not the organism was moving smoothly so that a trial could be begun. The stronger the background illumination, however, the more intense the CS has to be in order for the planarian to be able to sense its onset. One must also take great care that the trough itself is located between the source of the background illumination and the person observing the planarian's behavior. If the background illumination is overhead, for example, any movement that the observer makes will cast a shadow over the trough that will act as a CS of sorts. Furthermore, the intensity of the background illumination should be identical from day to day and from animal to animal. Therefore, conditioning studies cannot be performed adequately under "normal" daylight illumination, which varies radically according to the time of day and the weather. We have found it expedient to run our conditioning studies in a light-proof room, and to put the trough itself inside a large black box open at the front so that the observer can watch the planarian but with the background illumination coming from the rear of the box. Most sources of stray light and shadows are controlled in such an

arrangement.

Planarians are quite sensitive to temperature fluctuations, and the hotter they are, the more responsive they are to the onset of light. In general, planarians that one is likely to encounter in the United States survive best at temperatures between 50° and 70° F. Both the CS and the background illumination will, of course, act as heat sources, and a special effort must be made to insure that the temperature of the water in the trough is maintained at a relatively constant state, and that this temperature does not exceed 70° F. Since the CS is usually on for only a few brief seconds, it will have little effect on the water temperature in the long run. The source of the background illumination is, of course, another matter entirely. The onset of the CS will provide thermal stimulation to the planarian as well as visual stimulation; indeed, it is something of a question as to whether the planarian utilizes the change of temperature or the change in illumination as the real CS in such a situation. Rather careful studies by Westerman (see bibliography) suggest that light is by far the most important component of the CS. In any case, both the visual and the thermal stimulation should be the same from one trial to the next and it should matter little (for all practical purposes) which stimulus the animal becomes conditioned to.

Several other types of stimulation have been employed both as the CS and the UCS in various studies on conditioning. Planarians are sensitive to vibratory stimuli; weak vibration can therefore be used as a substitute stimulus for light as a CS, while a very strong vibration can be used as a UCS. However, planarians adapt very rapidly to strong vibration so it is probably a less useful UCS than is electric shock (to which the animals adapt but slowly). We have, however, successfully conditioned planarians using light as the CS and vibration as the UCS. A simple means of delivering vibration as a stimulus comes from attaching a doorbell or a buzzer of some kind to the conditioning trough itself; the difficulty with such a procedure is that one has little if any control over the intensity of the vibration. One can also use the offset of visual stimulation as the CS. Several years ago we trained a number of planarians as follows: a light above the conditioning trough was on continuously except at the beginning of a training trial, at which time it was turned off for a few seconds. After the animal had been shocked, the light came on again. In this situation, we found it expedient to use a fluorescent bulb that delivered considerably less heat than would an incandescent bulb. C. D. Griffard was able to condition his planarians using the onset of a current of water as a stimulus (see bibliography). All things told, however, the onset of light probably is the easiest and most reliable type of CS and electric current is probably the most reliable and easy to use UCS.

B. Mazes

The first person to achieve successful maze learning in planarians was probably Edward Ernhart of Washington University in St. Louis who, in 1959, reported that he had been able to train his planarians in what is called a 2-unit T-maze. Ernhart also reported that after the animals had been trained, when he cut them in half transversely (across the middle) and allowed each half to regenerate into a complete organism, both the head and the tail regenerates showed excellent and statistically significant retention of the prior training. Ernhart's results, of course, both confirmed and extended the prior findings of McConnell, Jacobson and Kimble. The use of mazes in experiments on animal learning has been standard since the American psychologist Small introduced them many decades ago. The usual maze is shaped like a capital T; the stem of the T is the "starting alley" into which the animal is put at the beginning of any trial. When the animal moves along the stem, it eventually encounters the two cross-arms extending at right angles from the stem. In some experiments, each cross-arm is painted with a distinctive color

or pattern and both cross-arms are different in color or pattern from the starting alley. The experimenter usually designates one of the cross-arms as being "correct" and the other as being "incorrect." If the animal enters the incorrect arm it is punished (or at least not rewarded) and usually is removed from the incorrect alley and placed again in the starting alley and forced to run another trial. If the animal enters the correct alley, it is typically given a reward (or at least not given punishment) and is usually removed from the maze for a period of time before being given another trial.

Another common shape for simple mazes is that described by the capital letter Y, in which all three alleys are the same length, meet at a central point, and form 120° angles with each other. Multiple-unit mazes may be constructed by attaching several simple T- or Y-mazes together. Schematic drawings of these various kinds of mazes appear in Figure 8.

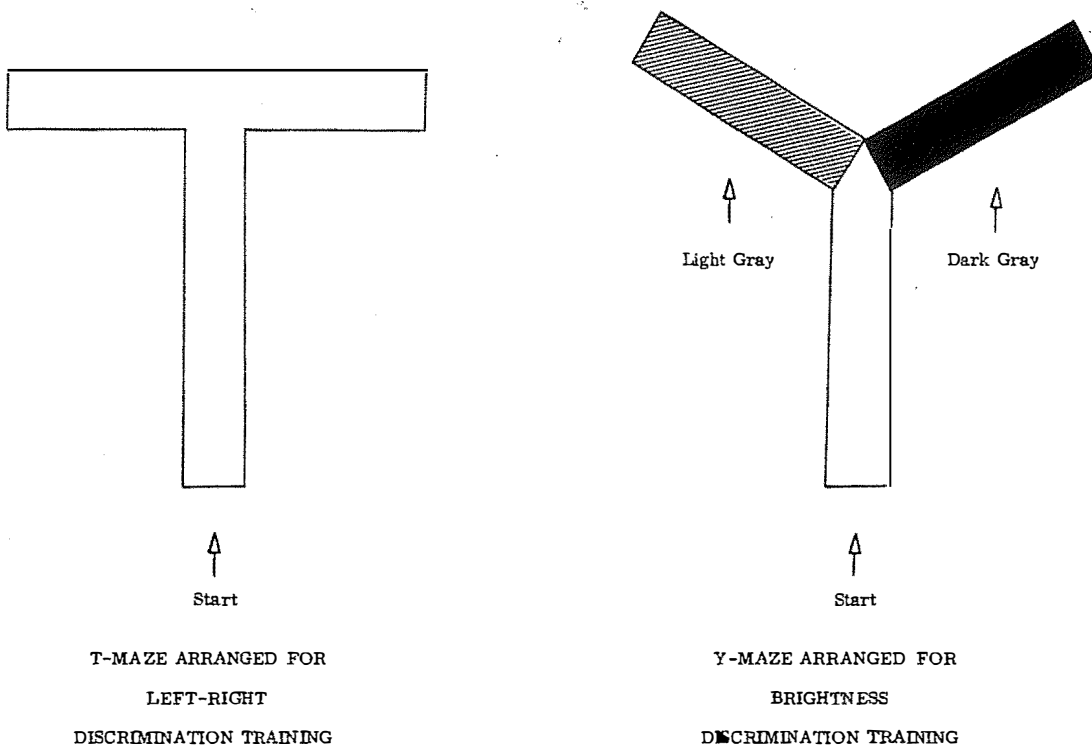


Fig. 8

Animals such as planarians learn to go the "correct way" in a maze because they are rewarded for doing so or are punished for choosing the incorrect route. It is up to the experimenter, however, to make certain that the task is as easy as possible for the animal and the experimenter must "see the world as the worm does" if he is to design a successful piece of apparatus. For example, all mazes for planarians should be either semi-circular or V-shaped in cross section; a maze which is U-shaped simply will not work. As the planarian crawls along on the bottom of a semi-circular or a V-shaped maze, it is forced to remain pretty much in the center of the alley (particularly if the width of the alley is not much greater than the width of the planarian itself), and when the animal reaches the choice point, it is forced to turn either left or right. In the case of a U-shaped maze, however, the animal may either crawl down the center, staying on the bottom of the maze, or it may crawl along either the left or the right hand wall. If the planarian is crawling on one of the walls when it reaches the choice point, it is not

forced to turn left or right but rather is forced to turn either up or down. In such circumstances, planarians usually cling to the wall on which they are crawling and ignore the fact that they have arrived at a choice point. Clearly, then, the more distinctive the experimenter makes the choice point, the more likely it is that the planarian will learn the maze readily.

There are two more or less standard procedures that one follows in training animals in a maze: either one teaches the animals always to go to the right or always to go to the left, or one trains them to approach a given stimulus (such as the white alley) no matter which side of the maze it appears on. If one uses "place learning" in a maze, then it is probably best for both the right and the lefthand alleys to be as identical as possible. If one wishes to train the animal to go to a distinctive cue (stimulus) then one must have two mazes, one of which is the mirror image of the other. For instance, suppose the left arm in a T-maze is painted white and the right arm is painted black and we wish to train the planarian to go to the white. If the white arm always is on the left, and the planarian learns the task, can we really say that it learned to approach white? No, because the planarian could just as easily have learned always to turn left in the maze. Therefore, we must have an identical maze in which the white arm is on the right and the black arm is on the left; during training the two mazes are randomly alternated.

What sorts of stimuli can be used in a simple maze? Most experimenters have used black arms and white arms since the planarian is quite sensitive to visual stimuli. However, Jay Boyd Best trained planarians in a maze in which one arm had a smooth surface and the other arm had a very rough surface. Obviously any two different stimuli which the animal can detect can be used as cues in a maze. There is, however, one problem that one has to face, particularly when using such distinctive cues as white and black. Under most circumstances, planarians tend to be "negatively phototaxic" (that is to say, they have a strong tendency to avoid light and to approach darkness). In a brightly lighted room, planarians will almost always pick the black arm of a T-maze and it is very difficult to train them to reverse this innate preference. One wishes to choose the stimuli for a maze in such fashion that naive (untrained) planarians will typically pick one arm just about as often as the other. Therefore, after the maze is designed and built, one typically tests a large number of animals in the maze to see what their naive preferences are. Unless the animals pick both sides with about the same frequency, the maze should be redesigned. For instance, we often find it more useful to use light grey versus dark grey in a simple T-maze since the behavior of naive planarians is much more random in such a maze than if we used a white and a black arm. By adjusting the intensities of the colors in the arms and the intensity of the overhead lighting, one can usually arrange the situation such that untrained animals will show relatively random behavior.

We have found it of some value to give each of our planarians at least 10 "preference trials" in a T-maze before actually instituting training (that is, before rewarding or punishing the animal for making a choice). If the maze is well designed, some of the animals will show a slight preference for white, some a slight preference for black, and some will go five times to the white and five times to the black (although the total number of black and white choices for all of the animals taken together will be about the same). If a planarian has shown a preference for white, we then make the black arm the "correct" arm during training, and vice versa for the animals that have shown a preference for black. Of the animals that have shown no preference at all, half are trained with white as correct and half with black. The "criterion of learning" in a simple maze experiment is usually 9 correct choices out of any 10 consecutive trials. One may, of course, adopt a more stringent criterion (such as 19 out of 20) if one wishes to do so.

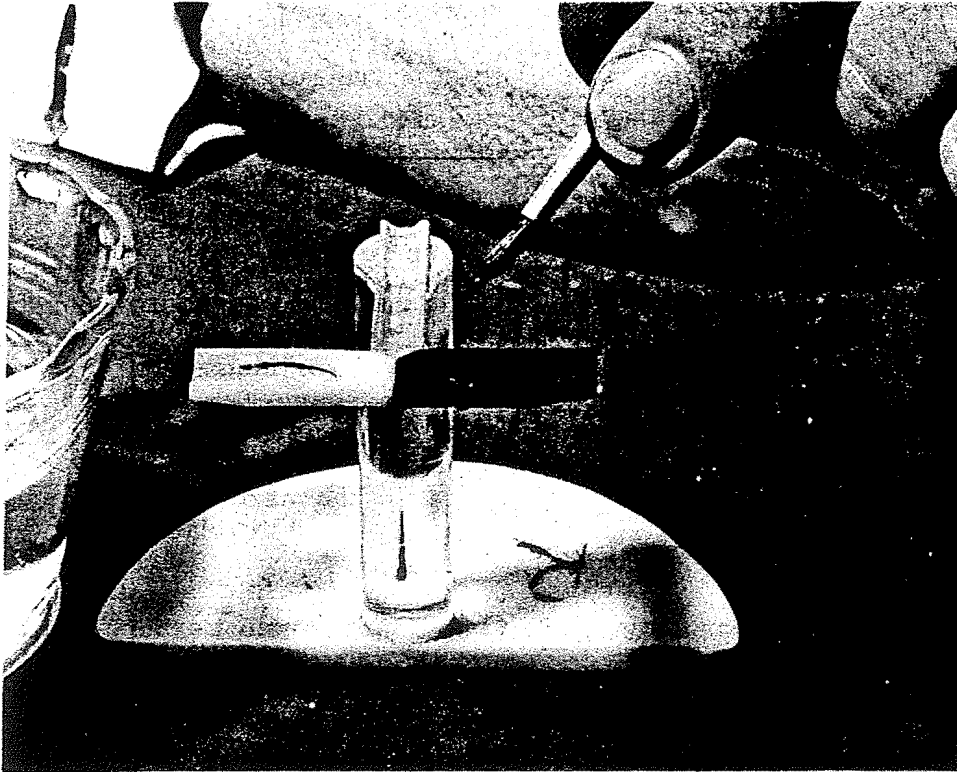


Fig. 9. A T-maze for planarians.

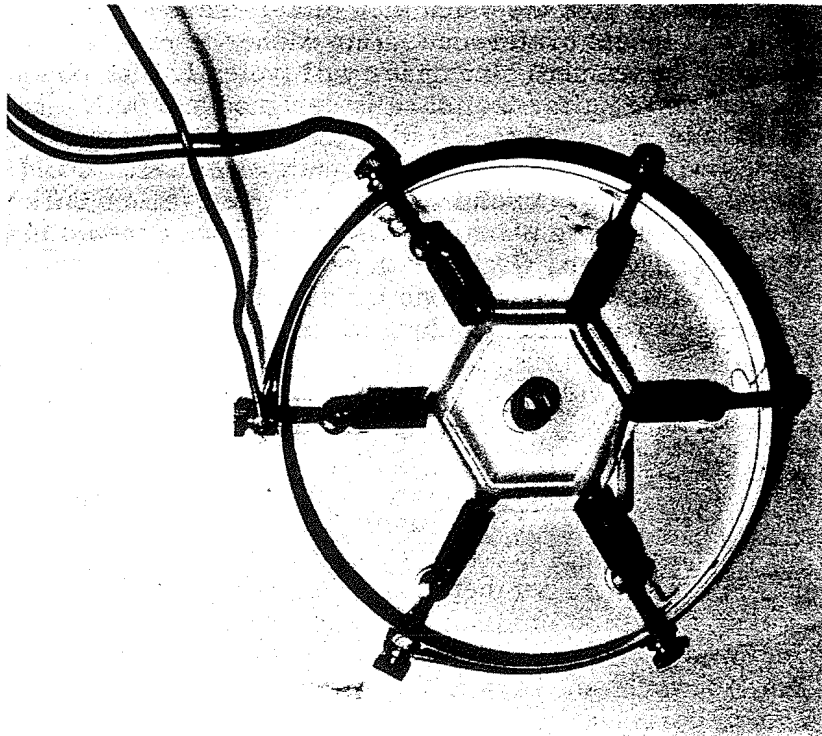


Fig. 10. A hexagonally-shaped planarian maze.

Since planarians occasionally turn around in a maze, it is necessary to set up a "response criterion" in order to judge when the animal has actually made a choice. One may either draw a line a few millimeters from the choice point on both the right and the left arms of a T-maze and decide arbitrarily that when any part of the animal has crossed this line, the animal has "chosen" that arm; or one may decide that when the planarian has moved at least half of its body into one arm or the other, it has "chosen" that arm. As the planarian begins to learn its way around the maze, it will often show what is called "vicarious trial and error" behavior at the choice-point--that is, as it reaches the choice point, it will poke its head first in one arm, withdraw its head, poke its head in the other arm, withdraw its head, and continue this behavior for some time before it actually commits itself to entering one of the arms. Unless the experimenter adopts a firm response criterion and keeps to it, he is likely to "fudge" in scoring such responses.

What sorts of rewards and punishments can be used in maze situations? Since a reward is usually defined as any situation or stimulus an organism will approach or do nothing to avoid, and since a punishment is usually defined as any situation or stimulus an organism will avoid or do nothing to approach, it might seem that one could use a great many different types of rewards and punishments with planarians. Theoretically this is true; practically it is not. In maze studies using higher organisms, the animal is typically given a positive reward such as food when it makes the correct choice; such a procedure is indeed difficult to employ with planarians. If one has food waiting for the animal at the end of a given arm, small particles and chemicals from the food will disperse through the water very rapidly and when the animal reaches the choice point, it has but to "follow its nose" to reach the goal. In such situations, planarians (as well as white rats and other organisms) appear to pay little attention to the stimuli in the maze--hence it is difficult to say that any real learning occurred. Jay Boyd Best overcame this problem by running his planarians in a dry maze and, if the animals chose the correct arm, rewarded them by filling the maze with water. We have typically trained our animals in simple T-mazes by using a combination of punishment and reward. Planarians typically do not appreciate being removed from their home bowls and put into the confines of a small maze; indeed, one might say that they show a certain anxious desire to return to more familiar and comfortable surroundings. Therefore, when our planarians make a correct choice (in a water-filled maze) they are immediately returned to their home bowls (using the elevated T-maze shown in Figure 9, we tilt the entire maze into the water and the planarian slides gleefully out of the correct arm back into its home bowl where it is allowed to remain for several minutes before being given its next trial). If the planarian makes an incorrect choice, it is picked up from the incorrect alley and started over again and does not get back to its home bowl until it makes a correct choice. In the hexagonally-shaped maze shown in Figure 10, we give the animals an electric shock whenever they enter an incorrect alley but do not return the animals to their home bowls until after they have run a set number of trials.

The difficulty with single-unit T-mazes is that one must handle the animals a great deal, particularly at the beginning of training when the planarian is making a significant number of incorrect choices. A hard and fast rule of planarian psychology is this--the less you handle your animals, the healthier and happier they will be. In order to decrease the amount of handling that the animal must receive per trial, we have recently switched to using a continuous Y-maze first designed by Kiki Roe, of UCLA, shown in Fig. 10. At the start of a day's training, the planarian is put in the center tract of the hexagonally-shaped maze and allowed to wander in either direction until it reaches one of the six choice points; whereupon it must choose either to continue in the white center track or to enter one of the black arms radiating from the center hexagon. If the planarian enters the black alley, it is given a brief burst of electric shock; if it stays in the white center track, it

avoids a shock. Each time the planarian crosses a choice point, we consider that it has run one trial. The beauty of the hexagonal maze is that it makes no difference whether the animal runs in a clockwise or a counter clockwise manner, nor how many times it reverses direction--the spatial relations obtaining between the black and the white arms are always the same no matter from which direction the animal approaches the choice point. In addition, from one direction the black arm will be on the left; from the other direction it will be on the right. Electrodes are buried at the ends of each of the six black arms radiating from the center white hexagon and are so wired that by pressing one switch, any of the six electrodes can be made the anode. It is important to arrange the shock circuit in this fashion since planarians do not learn the hexagonal maze well if they are given cathodal shock whenever they enter one of the black arms. There is nothing magic about this finding; you may recall that earlier I pointed out that when a planarian is headed towards the anode and shock is applied, the animal's head contracts, while if it is headed towards the cathode, the animal's tail contracts. When a planarian turns out of the center hexagon into one of the incorrect black arms, and one delivers cathodal shock to the animal, its tail contracts and it is automatically propelled directly into the incorrect arm; if at this point it is given anodal shock, its head is knocked back out of the incorrect arm and the animal is more or less forced to make another choice. If one wires the electrodes in arms one, three, and five together, and the electrodes in arms two, four, and six together, a simple circuit can be arranged so that pressing on one button turns electrodes one, three, and five into anodes and two, four, and six into cathodes, while a second button will make one, three, and five cathodal and two, four, and six anodal.

As in the case of classical conditioning, one should adjust the shock level in the maze so that the organism is given just enough current to evoke a consistent reaction whenever it enters the incorrect alley. Resist the temptation to "over punish" your animals--when the planarian enters the incorrect alley in the hexagonal maze, it should be given no more than one or two brief bursts of shock and then allowed to crawl around without further molestation until it re-enters the center hexagon of its own accord. Under no circumstances should you shock the animal when it is actually leaving the black alley, or heading in that direction, or you will be punishing it for making the correct rather than the incorrect response.

The planarian maze should be designed with planarians (rather than rats) in mind. If the alleys are too large, or too long, or improperly shaped, the planarian will have a great deal of difficulty learning what it is you want to train it to do. The more quickly you give the animal its reward after it has made the correct response, or the more quickly you punish it after it has made the incorrect response, the sooner the animal will learn. Maze learning is typically a more interesting type of training to employ than is classical conditioning, at least as far as the experimenter is concerned; but it is also more challenging since the experimenter can make a great many more mistakes both in the design of his apparatus and in his handling of the animals than is usually the case in classical conditioning.

C. Other types of training situations

Although most of the published research on learning in planarians has involved either classical conditioning or maze learning, there are several other types of situations in which planarians can be trained. For example, both Lee and Best (see bibliography) have been able to train their animals to turn off a noxious (unpleasant) stimulus such as a bright light overhead by interrupting a much weaker and smaller beam of light that activates a photocell. In this sort of situation, the planarian is housed in a small glass chamber during training. A large electric light is located immediately above the chamber; projecting upward through the bottom of the chamber is a tiny beam of light which is aimed at a photocell. Any time

the planarian crawls across that part of the bottom through which the tiny beam of light projects, the beam is interrupted (as you would interrupt the beam of a flashlight by putting your hand in front of it) and the photocell records the fact that the beam was broken. At the beginning of a trial, the bright light overhead goes on, prompting the planarian to movement. If the worm breaks the tiny beam of light, the photocell not only records this fact but also turns off the bright overhead light for 15 minutes. The number of times the planarian interrupts the tiny beam of light both when the overhead light is on and when it is off is recorded automatically in this situation. Both Lee and Best report that planarians appear to be capable of learning to interrupt the tiny beam of light in order to turn off the noxious stimulus. The good point about this type of experiment is that all the recording of the animal's responses is automatic. There is little likelihood that "experimenter bias" will influence the results. Unfortunately, this sort of experiment (which is usually referred to as "operant conditioning" since the animal learns "to operate on its environment") requires a great deal of sophisticated electronic apparatus; indeed, the cost of setting up just one of these "operant conditioning apparatuses" may run as high as several thousand dollars. A schematic drawing of Lee's apparatus is shown in Fig. 11.

C.D. Griffard (see bibliography) has devised two different conditioning procedures, one involving the onset of a current of water as a stimulus, the other involving the use of a moveable electrode to force planarians to turn in a certain direction. Both of these procedures are quite interesting in and of themselves but require a great deal more apparatus and scientific sophistication than do the maze studies and classical conditioning experiments described above.

The first American to train flatworms successfully was H. B. Hovey, who reported in 1929 that he had been able to train a marine flatworm in the following fashion. The onset of light typically will cause a motionless flatworm to begin moving; however, a tap on the animal's snout will usually stop the animal. Hovey brought his flatworms out into the light and, as soon as they began moving, tapped them on the snout gently. After several such taps, the worm would typically stop moving altogether. It would then be returned to the dark for a period of time and later brought out again for another series of trials. Hovey reports that the number of taps necessary to prevent all movement in the animals decreased markedly as training progressed. The use of various control groups assured Hovey that his results were not due to fatigue of or some injury to the animals. Although Hovey's experiment is a "classic," I cannot recommend its use if long term retention of the learning is desired. The reversal or "unlearning" of a strong innate reaction, such as Hovey obtained, is not only hard to achieve, but usually is quite short lived.

A rather different type of "training situation" is that employed by Shinkman and Hertzler and by Tricia Lee (see bibliography). If planarians are forced to make several right-hand turns in an apparatus such as they shown in Figure 12, they have a tendency to turn left when offered the opportunity to do so at the choice point of the T. In general, the more forced turns they must make before being given a free choice, the stronger the animals' tendency to go in the opposite direction. These results are similar to those found with a variety of other organisms, ranging from paramecia to white rats, and are of considerable theoretical interest. I cannot recommend this type of experiment to the average beginning worm runner, however, since each animal is typically given only one trial in the apparatus (and a great many animals must be run in order to get valid results) and since the tendency to turn in the opposite direction is usually "forgotten" almost as soon as the animal is removed from the apparatus. In short, this is not the sort of study one wishes to perform if one has but a limited number of experimental animals or if one wishes to study long term retention.

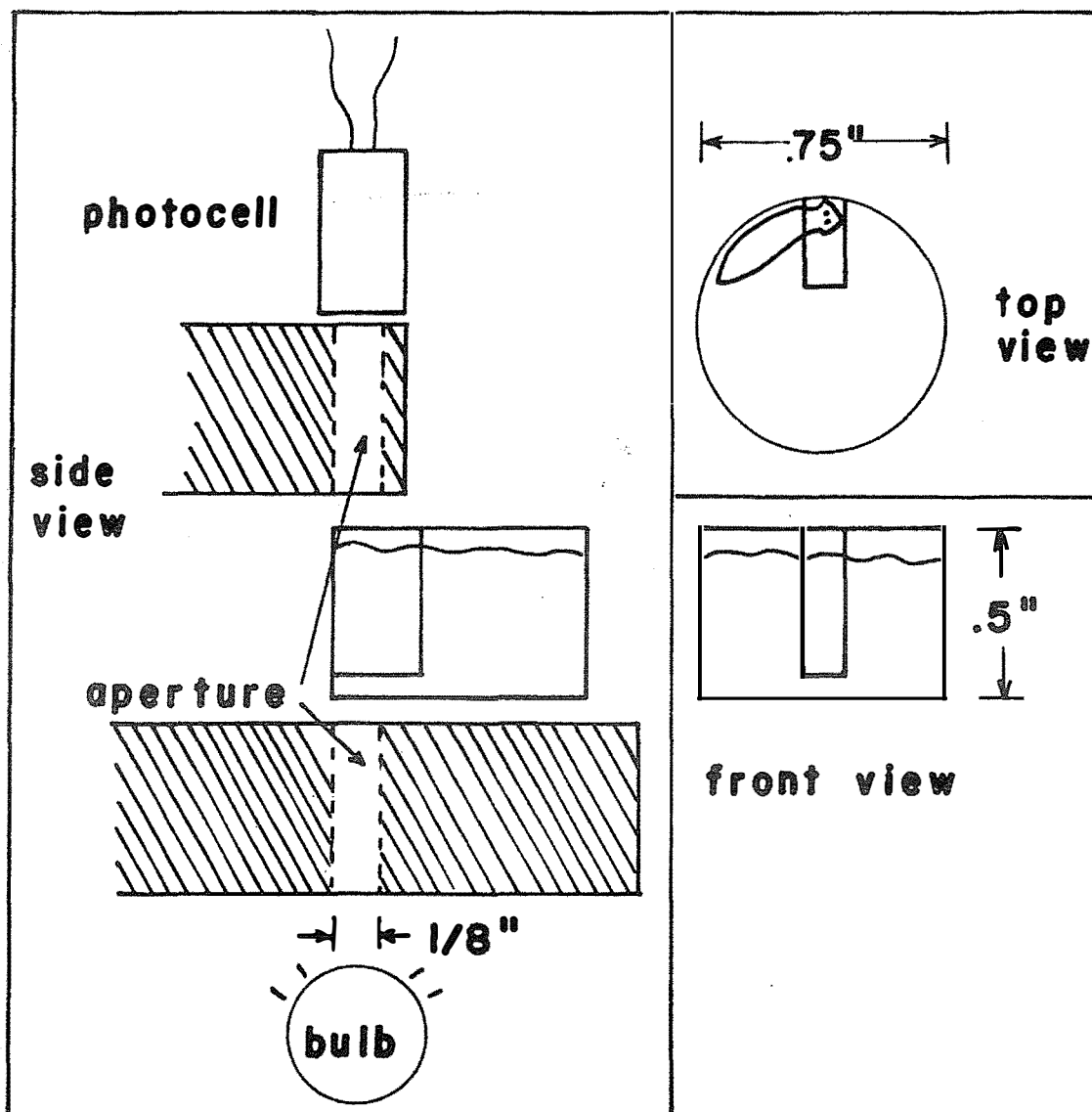


Fig. 11. Schematic drawing of photocell apparatus.

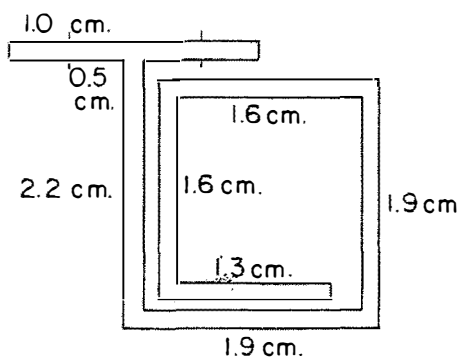


Fig. 12. Maze apparatus.

Oddly enough, one of the most convincing studies on planarian learning ever performed appeared in the literature 45 years ago and is probably also the first study of any kind clearly to demonstrate learning in planarians. This experiment was performed by the Dutch biologist P. Van Oye, and appeared in 1920. Since it was printed in Van Oye's native tongue and appeared in a little-read European journal, it was not generally known until Roman Kenk called it to our attention a few years ago. Most fresh-water planarians cannot swim--they must always be in contact with some surface in order to move. Thus they typically restrict their activities to the bottom and the sides of any container in which they are housed. On occasion, however, they can be seen to crawl upside down on the meniscus or surface tension of the water itself. Van Oye trained his animals to find a rather unusual route to food, and did so in the following fashion. First he put ten planarians in a glass container filled with water to a height of 7 cm. Then he hung a piece of meat on a metal wire and suspended the meat in the water so that it was 3.5 cm. from both the top and the bottom. Since the planarians could not swim through the water to reach the meat, the only way they could get to the food was by crawling along the bottom, up the sides, out on the meniscus until they hit the wire, and then crawl down the wire 3.5 cm. to the meat. Van Oye placed the meat in this position for several days and found that none of his animals ever reached the food. Then, several days later, he put the food on the surface of the water rather than 3.5 cm. below it. Now the planarian could find the meat simply by crawling out on the meniscus until it reached the food. Under these circumstances, it took but a few days until almost all the animals were reaching the meat each trial. Now Van Oye began lowering the meat a few mm. at a time. The planarians still reached the meat with great success, although Van Oye had soon lowered the meat to the point where they had to crawl down the wire a couple of mm. to get to the food itself. By moving the food lower and lower into the water on successive trials, Van Oye eventually trained the planarians such that they were reaching the food even though it was now suspended 3.5 cm. from the surface of the water.

We have been able to repeat Van Oye's experiment quite successfully in my own laboratory using two control groups that Van Oye did not think to employ. The success of our replications of Van Oye's work quite clearly depends on the manner in which the animals are handled, however. The planarian's visual system is quite simple--it can detect light-dark gradients, but its eyes have no lenses and it cannot see shapes or objects in the same way that higher organisms do. Furthermore, the animal does not seem to possess the rather sophisticated sensory equipment needed to allow it to detect slight "up-down" differences as it moves around the container it is in. If the planarian is on the bottom of the container when the food is put into the water, it senses the presence of the food chemically, and may even be able to detect that the food is somewhere above it. But even if it has learned that it must "go up the side of the wall and out on the meniscus and down a wire" to get to this food, it probably has no way of finding the wall of the container except through random motions, it may well have difficulty in determining whether it is moving straight up the side of the wall or at an angle, and once it has reached the meniscus, and crawled out on the surface film, it can probably find the wire itself only through chance or random motions. It is obvious from this discussion that the larger the container is, the more difficult it will be for the animals to learn how to get to the food. Furthermore, the closer to the center of the container one places the food, the more difficult it will be for the planarian to find the wire by random movement after it has crawled out on the meniscus. Realizing this fact, Van Oye inserted the wire close enough to one side of the container so that the animal could find the wire readily but not close enough so that the animal could reach out from the side and crawl directly on the food without first crawling all the way up to the top and out on the meniscus. One may make this learning task much more simple for the planarian by marking the side of the container at which the food will be presented with a characteristic color of some kind--in our experiments,

we mark the location of the food by putting a colored stripe up the side of the container as a trail which the animal may follow. We begin each trial by shaking all of the animals in the container into a small area opposite that point at which the wire will be inserted, a procedure which not only insures that all the animals begin each trial from the same point, but also activates those animals that were "asleep" when we were ready to begin a trial. Van Oye allowed his worms to feed on the meat as long as they wished on any given trial; we have found it better to let the animals feed for no more than a minute or two before shaking them off the meat and making them start over again. The hungrier the animal is, the more likely it is that it will seek out this rather unusual route to food. A hungry animal will therefore come back to the food several times during one "trial" if one does not allow it to feed too long once it has reached the meat.

We have used liver in most of our experiments but have learned that it must be handled with care. After the meat has been in the water for any length of time, it will begin to crumble and flake apart, and these particles of meat will fall to the bottom of the container. Sensible planarians will therefore wait underneath the meat until the particles begin to fall and will eat the particles off the bottom rather than climbing up the walls and down the wire to get to the main chunk of liver. One may overcome this sort of difficulty by searing the liver with flame before impaling it on the wire. One must also resist the temptation to hurry up the experiment by lowering the food in too large steps after the planarians have learned to reach the meat at the surface of the water. The first few steps downward should be no greater than one mm. , and the animals should be given several trials before the next step downward is made. Do not move the liver lower until all of the planarians seem to be reaching the meat with great regularity. After the meat has been lowered another step, one typically finds that the percentage of successful "hits" drops off sharply. If the percentage drops to zero, you have made too large a step and must pull the liver back up part way (at least) and begin again. The animals will also show a drop in percent of "hits" if they are overfed--do not run the animals more than a trial or two on each day (although each trial may well take 30 minutes or more) and do not run the animals more than 3 or 4 days a week. Again, shaking the animals off the food after they have been on it for a minute or two will help keep them hungry and therefore will help improve the speed with which they appear to learn.

The two control groups which we employed were the following: one group of planarians was given the same number of "trials" as were our experimental animals, but the liver was always suspended halfway between the bottom of the container and the surface of the water. After a great many trials, the animals in this group showed a slight improvement in their performance, but they still obtained only a small fraction of "hits" that the animals in the experimental group obtained. The second control group was treated exactly as the experimental animals were, but no meat was ever hung on the wire. We used this control group to determine how many times the planarians might be expected to hit on the wire strictly by accident. Needless to say, the animals in this latter control group showed no tendency whatsoever to increase their few "hits" from the beginning to the end of training. The animals in both control groups were given food at the bottom of the container for no more than two or three minutes following each trial to equate for the amount of food that the animals in the experimental group were obtaining.

The Van Oye experiment is a fascinating one and deceptively simple to perform. As with all other types of training, however, the more patience one has and the more feeling one has for the world as a planarian must see it, the more successful a worm trainer one will be.

V. Procedure

A. Pre-training

As I explained earlier, the wise experimenter will do everything possible to control everything in an animal's environment that might lead to variability in its behavior. Since it is rather a traumatic situation for an animal to be plucked from a large aquarium, dumped into a small glass bowl and shortly thereafter be removed from this new "home" and dumped unceremoniously into yet another new environment (the training apparatus), it probably is a good idea to set the planarians up in their small, numbered containers at least a week before an experiment begins. This gives the animal time to become habituated to living by itself in its new container before it undergoes the trauma of being trained. This also gives the experimenter a chance to look over each animal individually for a period of time to determine the animal's state of health. During this week, a few of the animals (particularly if one is using *D. tigrina*) will undergo spontaneous fission (that is, "drop a tail"); these animals should be discarded. Any animal whose behavior or general appearance suggests that it is not in top-notch condition should also be discarded before training is begun. The animals chosen should all be of approximately the same size (and if possible, of the same age) and certainly of the same species. You will probably get better results if you use animals that have been kept alive in your laboratory for several weeks prior to the beginning of training.

Planarians can be easily damaged and everything possible should be done to decrease the amount of handling they are given and to increase the gentleness with which they are treated. When transferring the animals back and forth from their home bowls to the apparatus, use a large camel's hair brush rather than a pipette. The brush itself should be several times larger than the planarians. If you must use a pipette (or a medicine dropper), make certain that the opening through which the water is to be sucked up is quite large--otherwise the worm will be shredded a bit as it is pulled through the opening. Planarians can be sucked up off the bottom of a glass bowl much more easily if you use a brush to shake them loose before using the pipette. Occasionally when one uses a pipette the planarian will cling tenaciously to the inside of the glass tube and will not be dislodged no matter how vigorously one pumps water back and forth into the pipette. Under such circumstances, one simply squirts all the water out of the pipette leaving the planarian high and dry. In less than a minute the planarian will begin to move in search of water, at which time it can readily be dislodged from the tube.

A planarian can live for several months without eating and is typically more active when hungry than when satiated with food. If one's training schedule will not extend for more than a couple of weeks, it is probably unnecessary to feed the planarians during this period of time. If the training is to take longer than two weeks, one can feed the animals but no more often than once a week. We typically give our animals a good feeding several days before an experiment begins and then do not feed them for at least several days after the training has started.

In my opinion planarians learn best when they are kept on a normal diurnal light/dark cycle--that is when kept in a room with unshaded windows when they are not being run. If the planarians are kept in total darkness for any length of time, they become hypersensitive to the light and will show a much higher naive response rate to light in a classical conditioning situation and a much stronger preference for the black arm in a maze situation than will animals that have been kept under normal lighting conditions. If for any reason it is absolutely necessary to house your animals in total darkness before training begins or between training sessions once the experiment has begun, give the animals at least fifteen

minutes of habituation time in a dim light before putting them in the apparatus. If you keep your animals in a room with unshaded windows, make certain that direct sunlight does not fall on them at any time on the day.

Planarians, like students, tend to be more active at night than they are during the daytime; for this and other reasons it is probably a very good idea to run your animals at the same time of day every day.

B. Habituation

It is probably an anthropomorphic truism that "the happy worm is an educable worm," but what can one do to make a worm happy? Aside from leaving it entirely alone or returning it to the stream from which it was taken, the best that one can hope to do is to make the animal as comfortable as possible in its present surroundings and to make sure that the training situation itself is as unstressful as it can be made. Planarians secrete mucus as they move, leaving behind them a trail of slime as they glide along. Very recent and as yet unpublished data from my own laboratory indicate quite clearly that planarians become very distressed and disturbed when they are put into any container or piece of apparatus that is not coated with a layer of slime. In a well-cleaned maze, for example, planarians will almost invariably pick the black arm and will learn only very slowly and reluctantly to pick the white arm instead. If the maze is coated with slime, however, the planarian will usually pick the white arm about as often as the black. A planarian put into a clean conditioning trough will show a much higher random response rate and will show much greater day-to-day variability in its behavior than if the trough is coated with slime. It does not matter whether the slime was laid down by one animal or a hundred--the results are the same. Apparently the presence of slime in a piece of apparatus makes a planarian feel "more at home." If the apparatus is clean, a planarian will not feel "comfortable" until it has itself coated the apparatus with slime. For this reason, we always keep on hand a bowl of untrained planarians that are put into the apparatus from time to time to "re-slime" the trough or the maze. Particularly after any piece of apparatus is cleaned, these "slime-providing animals" should be allowed to roam freely over the apparatus for several minutes to lay down a coating of mucus before an experimental animal is trained in the trough or maze. Be sure to change the water between animals or after the slime-providing planarians have been removed--the important components in the mucus are apparently not particularly water soluble and will remain in the apparatus if you empty the water out very gently.

In the case of classical conditioning, each experimental subject should be allowed at least 3 minutes of "habituation time" in the trough before training is started. When you first put a planarian into a conditioning trough, its behavior will usually be somewhat disturbed for a few minutes. Only when the animal settles down and is gliding smoothly along the bottom of the trough should the trials be started. The pre-training trials of "light only" that one gives to planarians in a classical conditioning situation and the "free choice" trials given in the maze also serve the purpose of allowing the animals to become accustomed to the apparatus before training actually starts.

C. Training.

In setting up a classical conditioning experiment, one must decide not only what stimuli will be used but also how the stimuli will relate to each other in time. For example, in our early conditioning experiments, we typically turned the CS (light) on for three seconds; during the final second, the UCS (shock) was also turned on. Only those responses which occurred during the first two seconds of the trial (prior to the onset of the UCS) were scored.

As soon as the animal was again gliding in a straight line, another trial was run. The average time between trials was around 20 seconds. The planarians were given 150 or more trials as rapidly as possible, usually within a time span of two hours or less. While planarians will show learning under these circumstances, their behavior is much more variable and it takes them many more trials to reach any set criterion of conditioning than if the trials are spaced out over time. Learning in planarians (as in all higher animals) seems to be more stable if the animals are given but a few trials a day, if the time period between any two successive trials (which is called the inter-trial interval or ITI) is at least a minute or more, and if the animals are run no more than three or four times per week. We usually give our planarians no more than 25 trials per day and run them on a Monday-Wednesday-Friday, or Tuesday-Thursday-Saturday schedule. The animals are fed either on Friday or on Saturday and the food is removed and the water changed a few hours afterwards.

Planarians are fairly sluggish animals and do not always respond rapidly to the onset of a stimulus. Thus, while in the past we have turned the CS on for only two seconds prior to the onset of the UCS, we have recently increased this time span to three or even four seconds with excellent results. If this time span is decreased to one second or less, the planarian simply does not have time to give a response to the light before the shock comes on, and little evidence of learning occurs.

In simple T-maze experiments, the animals should probably be given no more than 10 to 15 trials a day since they must be handled during each trial. I recommend giving the animals at least two or three minutes rest between trials. If one is running several animals at once on a T-maze, a simple procedure is to run one animal for a trial and, after it has been rewarded by being put back in its home bowl, the next animal is run, etc. Try to arrange things so that if the first animal is being trained to go in one direction, the second animal is trained to go in the opposite direction, a procedure which would control for any tendency the animals might have to follow each other's slime trails in the maze. In the case of the hexagonal maze, since the animals are not handled between trials, one may give the planarians 25 or 30 trials per day. We find it wise, however, to run the animals in the hexagonal maze no more than two days per week.

If an animal ceases to move during a trial, it can be prompted to motion by touching it gently on the tail or by disturbing the water in its vicinity with a pipette. Keep track of the number of times that each animal had to be prodded to motion. If an animal refuses to move at all, even after repeated proddings, it should be returned to its home bowl and given several days to rest.

Occasionally an animal will turn upside down and crawl on the meniscus during a training trial. If this happens during maze learning, force the animal to turn right side up immediately. If it happens during classical conditioning, do not disturb the animal (unless it remains upside down for a very long period of time) but under no circumstances run a training trial while the animal is crawling on the meniscus.

D. Avoidance and Escape Learning

When psychologists speak of "conditioning," they usually are referring to "classical or Pavlovian conditioning." However, the term is often used as a synonym for any type of learning whatsoever. Most of the time when some other form of conditioning than classical conditioning is being referred to, however, the psychologist will modify the term to give his listener a clearer idea of what type of training he has in mind. In "classical conditioning," for example, the CS precedes the UCS, while in "backwards conditioning" the UCS precedes the CS, and in "pseudo-conditioning," the CS and the UCS are presented

to the animal randomly, but unpaired, and are usually separated in time by at least 30 seconds or more. Many learning theorists hold that backward conditioning and pseudo-conditioning techniques should not lead to any learning on the part of the animal, a viewpoint hotly contested by other theorists.

In "escape conditioning," only the UCS (typically a noxious stimulus such as electric shock) is presented; the animal can escape this unpleasant situation by making some action such as pressing on a lever or leaping a hurdle to get into a different compartment in the training apparatus. If a warning signal (a CS, of course) precedes the onset of the UCS, and if the animal may prevent the onset of the UCS by making some response, one speaks of "avoidance conditioning." In classical conditioning using light as the CS and shock as the UCS, the animal is shocked whether or not it responds during the few seconds that the light is on prior to the onset of the shock; in avoidance conditioning, if the planarian responds to the light, the light is immediately turned off and the animal is not given any shock on that trial.

We succeeded in training a few planarians in 1957 using an avoidance conditioning paradigm with light as the CS and shock as the UCS, but for a variety of reasons switched back to classical conditioning shortly thereafter. Quite recently (1965) Ernhart at Washington University informed me that he had compared the effects of classical versus avoidance conditioning in planarians and got much more stable and less variable behavior when using avoidance than when using classical conditioning (private communication). Ernhart's results are quite interesting, since this is the sort of finding that one typically gets with many higher organisms. The difficulty with using avoidance conditioning in planarians (or any other organisms) is this--as the animal approaches criterion, it seldom if ever receives the UCS, a condition which theoretically should rapidly lead to experimental extinction.

The jargonish word "extinction" often confuses the non-psychologist, probably because the psychologist typically misuses the term himself. Extinction refers to an experimental procedure which is designed to achieve the opposite of training--that is, the tearing down rather than the building up of a conditioned response. If the pairing of the CS and the UCS leads to an increase in the probability that the animal will respond to the onset of the CS, then if we give the organism a great many trials of the CS alone without presenting the UCS, we should decrease the probability that the animal will respond to the CS. Suppose we have trained a planarian to go to the black arm in a T-maze by rewarding it somehow whenever it made the correct turn at the choice point; after it has learned this habit, we can "untrain" the animal by giving it a great many trials in the maze in which it does not receive reward if it chooses the black arm. If we classically condition a planarian by pairing light and shock, and the animal eventually comes to respond to the light 90% of the time or more, we can then institute "extinction training" by presenting the light for a great many trials and never giving the animal shock no matter what it does. In truth, we have "extinguished the conditioning." A psychologist would likely refer to this as "extinguishing the animal" although he really means that he has extinguished the habit. Most organisms (including planarians) that have been classically conditioned to a set criterion and then put on extinction training will rapidly drop back to their naive response rate to the CS (extinction usually takes fewer trials than does conditioning). In better than 90% of the learning experiments involving extinction reported in the scientific literature, the experimental animals were trained to some arbitrary criterion and immediately upon reaching this criterion were at once put on extinction training. Under such conditions, animals usually show a rise in response rate to criterion during conditioning, and a drop off back to the naive response rate in extinction. Almost never do psychologists bother to give

their animals large numbers of conditioning trials after the organism has reached criterion. Oddly enough, planarians and most other organisms will often undergo "spontaneous extinction" after they have reached criterion even if one continues to give conditioning (rather than extinction) trials, a fact that has received scant attention from most experimental psychologists perhaps because it does not readily fit within their theoretical frameworks. Spontaneous extinction is also found in maze learning and, indeed, in almost every other form of training. For reasons not yet clear, planarians appear to undergo spontaneous extinction much more readily than do higher organisms, a fact that worm runners must constantly keep in mind.

There are many different methods for measuring whether or not learning has occurred. For example, one may measure the probability that a response will occur; in a well designed maze experiment, the probability that an animal will pick a given arm on any given trial at the beginning of training is 0.5, while at the end of training the probability has usually risen to at least 0.8. One may also measure the strength of a conditioned response (animals tend to respond more vigorously after they have been trained than before they have been trained), or the latency of responding (animals typically respond more rapidly to the onset of a stimulus after training than before training). One may measure the retention of learning over time by means of a "savings score"; if it takes a planarian 150 trials to reach a given criterion of conditioning the first time it is trained, and if one lets the animal sit without any further training for a month or two and then retrains the animal to the same criterion, one expects to find that the animal will take fewer trials to regain this same criterion the second time around than it did the first.

A rather crude measure of the strength of learning can be calculated from determining the resistance to extinction that an animal shows. Let us assume that the more strongly ingrained a habit is, the more difficult it will be to "extinguish" this habit (a dubious assumption at best). We could then train one animal by one method, a second animal by a second method, and institute extinction training for both animals after they had reached criterion. If it took longer for the first animal "to be extinguished" than the second, we could then assume that the first animal had "learned better" than the second.

Because these various methods of measuring learning apparently tap different aspects of the learning process itself, they seldom give the same results. Let me illustrate the difficulties by comparing classical and avoidance conditioning in the dog. Suppose we place our dog in a harness that suspends the animal in air with its legs hanging downward. We now attach an electrode to one of its front paws. The ringing of a bell serves as our CS, shock delivered to the paw serves as our UCS. If we pair the CS and the UCS in a classical conditioning paradigm, the dog will rapidly begin lifting its paw as soon as the bell rings rather than waiting for the shock to force it to make this response. Learning should take no more than 10 or 20 trials. If we now institute extinction training (that is, sound the bell without ever delivering shock), the dog will rapidly stop lifting its paw when the bell rings; extinction should take no more than 10 to 15 trials. Now let us change the apparatus--instead of attaching the electrode directly to the dog's paw, we will put an electric grid under the animal so that as it hangs in the harness, its paw just barely touches the grid. Now the animal can obviously avoid the shock by lifting its paw when the bell rings; it will do so often in fewer trials than were needed for classical conditioning. Now we institute extinction training--that is to say, we turn off the shock entirely. The dog, of course, does not know that it will no longer be shocked for failing to lift its paw and indeed will continue to lift its paw "to avoid the shock" for hundreds and hundreds of trials after "extinction training" has been instituted. Poor stupid dog! Obviously avoidance training, although it took fewer conditioning trials, is stronger than classical conditioning if one looks at the extinction data. But suppose we modify the extinction procedure just a little; suppose we tie the dog's paw down to

the grid so that it cannot lift its paw no matter what happens. Now, if we sound the bell and do not give the animal shock, it will undergo "extinction" in five trials or so. Which set of extinction data shall we believe?

Why do animals undergo extinction? At a very simple level of analysis the answer seems to be this: Training usually involves the use of some kind of reward and build up of some connection between two different types of stimuli, or at least between a stimulus and a response. This is the positive factor in learning. But during training, negative or inhibitory factors are also building up; lower animals, like humans, tend to obey Zipf's law, and it is usually easier for an organism not to make a response than to make a response. In a classical conditioning situation, it helps the animal little to make a conditioned response--the beast will receive the shock no matter what it does, and most animals apparently are capable of learning this fact. Spontaneous extinction is then to be expected in any type of classical conditioning situation. Avoidance conditioning is a different matter entirely; if the UCS is made particularly noxious (as was the case in the experiment with dogs mentioned above) the animal can avoid a considerable amount of pain by continuing to respond to the CS indefinitely. In avoidance conditioning, unless the experimenter somehow "notifies" the animal that extinction training is now underway, the animal is likely to continue to respond for a long period of time. If the noxious stimulus is quite weak, this massive resistance to extinction typically does not obtain, and the animal's own innate lethargy soon forces the animal to omit a response to the CS, at which time it discovers that the UCS is no longer being presented, whereupon it typically "extinguishes" quite rapidly.

Planarians are very lethargic animals and often respond to any form of stimulation by contracting and refusing to move or to respond further. The more training a planarian is given in a short period of time, the more inhibitory or lethargic it usually becomes. Jay Boyd Best trained his planarians in a dry maze; if they chose the correct alley, they were rewarded by having the maze flooded with water. If they made the incorrect choice, they were forced to retrace their steps and were not given water until they had entered the correct alley. If they refused to run the maze at all, they would of course eventually die from desiccation. Best found that his animals typically showed random choice at the beginning of training; after they had been rewarded for picking the correct alley a few times, the probability that they would pick the correct alley on any given trial rose significantly. If Best continued to train the animals, however, they usually showed a dramatic reversal and began picking the incorrect alley almost all the time. Soon after this, the animals would refuse to run the maze at all and would have perished had not Best rescued them. That this dramatic reversal of choice and refusal to run the maze at all were not due to the operation of a "freudian death instinct" in planarians is shown by the fact that these phenomena disappeared almost entirely if Best gave his animals only a few training trials per day, with considerable time off between trials. We have confirmed Best's results many times in my own laboratory, and have likewise found that giving the animals too many conditioning trials per day leads to the same sort of result. We refer to this phenomenon as "conditioned inhibition" or "conditioned lethargy."

VI. Cannibalism

Anyone who wishes to perform studies involving "the cannibalistic transfer of training" in planarians will do well to remember the following facts. First, D. dorotocephala cannibalizes more readily than does D. tigrina. Second, all planarians will cannibalize members of another species more readily than members of their own species. Third, all planarians will cannibalize more readily if hungry than if satiated. Fourth, within a given

species, some planarians will cannibalize more readily than others even if all the animals have been starved for the same length of time. Fifth, once a planarian has shown cannibalistic behavior, it will very likely do so again the next time the opportunity is presented to it.

When we wish to undertake a study involving cannibalistic transfer of training, we typically select a large number of healthy looking specimens and starve them for a week or more. Then we chop up several other planarians and put the minced pieces in with the hungry worms. Some of the intact animals will immediately begin to cannibalize--these animals are removed from the bowl at once and are used as experimental subjects. Those animals that refuse to cannibalize under these circumstances are discarded. The cannibals are then typically starved for another several days before they are to be fed experimental or control subjects in the experiment proper.

If we are going to feed trained animals to untrained cannibals, as is typically the case, we usually feed the "victims" to the cannibals as rapidly as possible after the victims have finished their training. If the victims are trained to a given criterion, we remove them from the training apparatus the instant they have reached criterion and cut them into equal thirds with a razor blade. Each third of the victim is fed to a different cannibal. If the cannibal is "asleep" when the piece of trained victim is put into its bowl, it should be awakened by prodding it gently in the tail with a brush. The piece of the victim should be poked as close to the cannibal as possible. Usually the cannibal will almost at once seize the chunk of victim worm and devour it. Cutting the victim into thirds does not kill the victim, of course, and the pieces of the victim continue to move even after they have been put into the bowl with the cannibals; indeed, the pieces will often seem to be attempting to elude the cannibal. If the cannibal does not seem interested in eating, or if the piece of the victim worm is successful in eluding it, we usually perforate the piece of the victim with a needle or a pin several times, an operation which not only tends to slow down the victim but also releases into the water more of the "wound substance" that excites the cannibal's appetite. It is not enough merely to put the piece of the trained victim into the cannibal's bowl and hope that the cannibal will eat it--one must stand by watching to insure that the cannibalism has actually taken place, for the piece of the trained victim will occasionally "disintegrate" spontaneously in a matter of a few hours thus deluding one into thinking that the cannibal has in fact dined when it hasn't. A well starved cannibal usually has no difficulty in downing a third of an animal its size. If one minces the victims into pieces smaller than a third, one has considerable difficulty in determining exactly how much a given cannibal has been fed, not to mention difficulty in determining how much a given cannibal actually ate.

To the best of my knowledge, no one has performed a study to determine whether or not feeding cannibals the heads, middles, or tails of trained victims makes any difference in how much "learning" is transferred to the cannibal. In most of our studies involving cannibalism, we fed each animal three times or more and made certain that each cannibal ate one head, one middle, and one tail. Our studies also suggest that three feedings are as effective as 12 or even 24 feedings. It has been our practice to give the cannibals one or two feedings of "trained victim," then to wait at least 24 (usually 48) hours before we begin training. If we plan on no more than 25 trials per day, we typically give the cannibals additional feedings as training continues. In any case, one should probably wait at least 24 hours after feeding before giving the cannibals any training at all since it presumably would take at least that long for the "memory chemicals" that the cannibal has just devoured to be distributed throughout its body. Our own studies suggest that the best time to institute training is 48 hours after feeding, and that if one waits as long as 7 days after the feeding before starting the training, not much transfer is accomplished. But Capretta (see WORM RUNNER'S DIGEST, Fall 1965) got almost as much transfer when he waited 7 days before starting

training as when he waited but one day. As of the spring of 1965, we do not know the minimum amount of tissue that a cannibal must ingest for any transfer at all to take place, we do not know whether the more a cannibal eats, the more transfer one gets, nor which part of the victim's body is most effective in achieving transfer, nor how long it takes for the chemicals the cannibal has ingested to become effective, nor whether it is a specific memory that is transferred via cannibalism or merely an excitatory substance of some kind which speeds up learning in the cannibal, nor what the chemicals are that achieve the transfer (if indeed chemicals are responsible at all!). All we do know is that if an untrained cannibal eats a trained victim, the cannibal will, right from the start of training, be significantly superior to another cannibal that ate an untrained victim. Obviously, as scientists like to put it, "further research is needed in this area."

Anyone undertaking studies of the cannibalistic transfer of training in planarians (or studies involving injection or regeneration), should take particular care to guard against "experimenter bias" by utilizing "blind" experimental techniques (described above) and any other safeguards he can think of. "Transfer" studies that do not contain adequate controls are no longer acceptable for publication in most journals (including the WORM RUNNER'S DIGEST) and probably should not even be entered as Science Fair projects.

VII. Publication

It is customary, after one has performed an experiment or a series of experiments, to communicate one's results to one's colleagues. Indeed, it is often said that one experiments in order to publish (that is, to see one's name in print). Whatever one's motivations, publication of research findings is obviously a worthwhile endeavor. Most beginning scientists have little idea of how to prepare a manuscript for publication, however, so perhaps a few words of advice are in order.

Any scientific paper worth its salt is written with a particular audience (and usually with a particular journal) in mind. Since, in the long run, the journal itself determines both the style in which one writes and the audience that an article will reach, the beginning writer is well advised to consider seriously first of all to which journal he intends to submit his paper. In general, the safest procedure is to hunt for the journal which has already published one or more articles of the same type or on the same subject that one intends to treat. Once a writer has selected his target journal, he should quite carefully read several recent issues of that journal (no matter how distasteful a task this may seem), select one or two articles that seem to be quite similar to the paper he intends to prepare, and then pattern his manuscript as closely as possible after these "model" papers. Most scientific journals contain at least some sketchy "advice to authors" somewhere in each issue of the journal, or publish a "Manual of Style" which may be obtained from the editor for a small fee. Most scientific articles are comprised of an introduction, a section on apparatus and procedure, a results section, and a discussion section. The article will also usually contain either an abstract or a summary and conclusion section.

The introduction typically begins with a general statement of some broad problem area within a given scientific discipline, a brief history of the problem and a survey of the research done in this area, and then narrows down to a tiny segment of the topic area itself. The introduction usually ends with a brief description of the experiment which was undertaken and a discussion of how this experiment might be expected to have pertinence to the topic area as a whole. The supposed purpose of the introductory section is to explain to the reader why this particular study was done; in fact, the introduction acts as "bait" to lure the unwary reader into plowing through the rest of the article.

In the apparatus and procedure section, one should (in theory, at least) describe what one did in such detail that anyone reading the paper should be able to repeat the experiment exactly if he so wished; this section is therefore quite extensive. In fact, it is almost impossible to replicate any given experiment merely from reading about it in a journal article; the author usually manages to omit several critical bits of information about his work simply because he is so familiar with what he did he cannot realize that anyone just beginning to work in this area would of necessity lack the author's extensive background. The major failure that most scientists have in communicating their results stems from this one fact--they know what they did, and they know what they mean by the technical terms they employ, but they do not realize that their audience typically does not share this knowledge. As a rule of thumb, put in the apparatus and procedure section every bit of information you can think of that would help explain exactly what you did. The editor will surely cross out much of what you have put down, but at least you made the effort! Remember that at a given point in time, no one really knows what the truly important variables in any scientific study really are, and two experiments which differ only slightly in detail may often yield quite opposite results; hence, include all details that you can.

The results section should include whatever charts, graphs or tables are necessary to make it immediately clear to the average reader precisely what the findings of the study were. If the behavior of two different groups is to be compared, one should be all means make the comparison statistical. To say that group A learned faster than group B is to tell your reader absolutely nothing worthwhile; to say that group A learned significantly faster than group B, and that such results would not be expected by chance alone more than 1% of the time, tells the reader exactly how much faith he may put in your findings.

In the discussion section, one pats oneself on the back for having performed such a crucial experiment and one explains away all the results that were not consonant with one's preconceived notions. Quite often in the introduction section, a scientist will set up an hypothesis which he intends to test in the experiment he describes. It is almost inevitably the case that in the discussion section he will point out how beautifully his hypothesis was confirmed. The introduction is, of course, written as if the hypothesis were made before the experiment itself was run; in fact, one often changes one's prior ideas of what the results of a given experiment would be after the experiment has been run. In such cases, one is careful not to state one's old ideas in the introduction if one can possibly keep from doing so; rather, one states one's findings in "hypothetical form" in the introduction--a device that insures that one's hypotheses will always be confirmed. An exception to this rule occurs when one is testing someone else's hypothesis in a given experiment; one confirms or disproves another scientist's hypothesis depending (usually) on how friendly one is towards the other scientist. At the end of the discussion section, one speculates about other studies which might follow from the one just described and concludes by stating that "further research is needed in the area."

Unless one is writing for publication in such a bizarre journal as the WORM RUNNER'S DIGEST, one should make every effort to eschew or avoid the use of humor in writing a scientific article. I say this in all seriousness. There is a strong tendency in scientific circles to distrust any article which smacks of "cleverness". It does help for an author to write well, however; the editor will surely be shocked if he can find no grammatical errors in a manuscript, and if he can understand from just one reading precisely what it is the author did and why he did it, but few editors will reject a manuscript out of hand for these reasons. Indeed, a clear, well-written article stands a much greater chance of being accepted for publication than a poorly written one does, if only because the former requires

considerably less work on the editor's part than does the latter.

All manuscripts should be typed on one side of the page only, double spaced, and submitted in duplicate. Most journals require that an article be read by a referee or critic prior to acceptance, and most editors will pass along the referee's criticisms when they write you telling you whether or not the manuscript has been accepted for publication. Almost all manuscripts require some revision before being published, the most common suggestion from the editor being that the author cut the paper down to half its present size. If an author does not agree with a referee's criticisms, he should say so loudly and strongly. But the author should be more interested in winning the war (that is, in getting the article published) than in winning any particular battle over editorial revision. The author might also remember that most editors have considerably greater journalistic skill than does the average author and that the scientific manuscript that could not be improved by good editing has yet to be written.

VIII. Afterthoughts.

A. What actually gets learned?

According to most psychological theories, when an animal is put in a training situation, it learns new habits or new responses to the environment in which it finds itself. But consider a simple T-maze in which a planarian is trained to enter the black alley. When it solves this problem, what can we say that it has learned? Has it done no more than to "acquire the habit of approaching black," or "the tendency to respond by turning right at a given choice point?" No training situation is probably that simple. A planarian in a maze first of all has to learn that something is expected of it--that it must perform in order to avoid the punishment or achieve a reward. It must therefore become habituated to the handling that it will receive before, during, and after each trial, it must become accustomed to the apparatus itself, to the training regimen and the feeding and diurnal lighting schedule, to eating a certain kind of food, etc. It must become sensitive to the new patterns of stimuli in the maze itself--that is, it must learn to notice that one arm is black, the other white, and that the stem of the maze is a neutral color. It will probably also learn how high the water level is in the maze, the width of the arms, the intensity of the light overhead, the temperature of the water (whether it is the same or different from the water in its home bowl), whether or not there is slime in the maze, the texture of the floor and the walls in the maze, the length of each of the arms, and perhaps a dozen or even a thousand other things about the experimental situation. We seldom bother to measure any of these forms of learning, being interested only in whether the animal turns right or turns left (and at that, not being interested in how the animal makes the correct response). All forms of learning probably include components such as "habituation" and "sensitization," although many psychologists speak as if "learning" were different than sensitization and habituation rather than including these types of behavioral modification. Because our theories of (and hence our understanding of) learning are quite simple, we typically take a simplistic viewpoint towards the behavior or organisms. But animals, even planarians, are not at all simple nor are their behaviors as uncomplicated as we sometimes like to pretend in scientific articles. The scientist who is more interested in his own prior theoretical preconceptions of what planarian behavior should be than in discovering what planarian behavior could be does a disservice not only to science, but also to his animals. If you attempt to train your animals in a given situation, and they do not appear to learn, blame people like me for not telling you how it should be done or yourself for not being bright enough to figure out what is wrong rather than assuming that the fault necessarily lies in the planarians. We do not know as yet how intelligent flatworms

are; we do have ample evidence, however, that the more we know about the animals, and the greater control we gain over their environment, the more easily we can shape their behavior in a wide variety of extremely complex situations.

B. The purpose of Science.

Underneath it all, science is a way of life just as Presbyterianism or bootlegging. Science is one of many possible methods of finding out the whys, wheres, hows, and what ifs' of the world around us, including our own behavior. But science is also a way of killing time and of earning money, a way of challenging one's intellect and of gaining the respect and admiration of one's peers. Science should therefore be a pleasant occupation, and one probably should not be a scientist if one could be happier doing anything else. Life is far too short a game for anyone to waste much time doing things simply because he's told they ought to be done, whether those "things" be playing baseball, listening to Bach, or running worms. Try worm running on for size; if you don't like it, the worms will know it even before you do. If you do like it, if it fascinates you as much as it does me (and hundreds of other people), if you think the whole business is really fun, then you are in luck--and so are the worms.

ADDENDA

This MANUAL was written during the spring of 1965. To keep the MANUAL as current as possible, however, we will revise it as often as is practicable.

November, 1965: Recent studies suggest that the contraction is a better index of learning in classical conditioning situations than is the head-turn. We have mentioned the reasons for this on page 32 (revised).

For a long time, I have been bothered by the fact that most animals do not maintain as high a level of correct performance in the hexagonal maze as they do in the T-mazes. It finally occurred to me that a planarian, in order to obtain a "perfect" score in the hexagonal maze, must move consistently clockwise or consistently counter-clockwise. A glance at the maze (pictured on page 40) will show why. Yet, when the planarian moves always in one direction, its innate tendency to alter its direction of movement (discussed on page 43) is working directly against its tendency to pick the correct alley at each succeeding choice point, and the more correct choices it makes in a row, the stronger the build-up of its tendency to pick the opposite (incorrect!) alley at the next choice point. This inherent weakness of design has now caused us to abandon the use of the hexagonal maze for most purposes.

A very important set of papers on planarian behavior and learning, published too late to appear in the Annotated Bibliography of this MANUAL, can be found in the Special Supplement to the British journal, Animal Behaviour, published late in the summer of 1965.

Section IV

GRAFTING TECHNIQUES FOR PLANARIANS

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Graft and transplantation experiments with planarians allow the investigator to ask the organism a nearly infinite variety of complex questions with a simple method. Two basic methods of grafting are to be described, both of which have been used successfully with Dugesia dorotocephala in the author's laboratory.

General considerations

Two points must be kept in mind when attempting graft or transplantation operations: first, the animal must be restrained from moving; second, the actual operation must be performed as rapidly and deftly as possible. Complete anesthesia of planarians is extremely difficult with conventional methods. The best method in our hands combines the anesthetic MS-222 (Sandoz Pharmaceutical), 0.03% in tap water, with cool (10-15°C) temperatures. Exposure to this anesthetic should not exceed 5 minutes. All operations should be done with the aid of a dissecting microscope. The safest way to transfer planarians is with a small artist's brush, not with a pipet. When transferring from a damp surface it is best to lift the worm with a small, flat spatula. A nearly ideal cutting surface for routine use is provided by a flat clean piece of soft pine placed on the stage of the dissecting microscope. It should be kept moist during use.

In the subsequent procedures, there will be need for Petri dishes with a layer of 2% agar poured in the bottom half. Some should be made with a 2 to 3 mm layer of the agar to be used as a physical substrate, and a few with a 1 mm layer. The thinner sheets of agar will be cut into narrow bands for the purpose of securing the graft until healing occurs. It is definitely advisable to use highly purified agar.

I. Implantation graft technique

Cohen (1964) has recently described a similar but somewhat more laborious method for the implanted type of graft.

A host and a donor worm are placed on the damp cutting surface. A hand-made pipet (see Figure 13) is used to punch out a hole in the host worm and the hole is filled with a piece of tissue from the graft-donor worm. The same pipet is used to prepare the graft tissue, thus ensuring a snug fit of the graft.

In detail, the procedure is as follows:

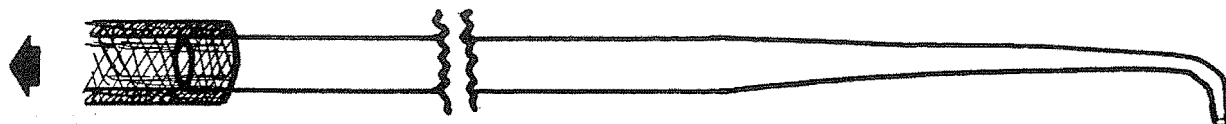
1. Prepare the graft site in the host worm by punching out the appropriate area with the pipet. Discard the tissue plug by blowing it out of the pipet. Transfer the host with a spatula to the agar-lined surface of a Petri dish.
2. Punch out the region to be grafted from the donor worm and with it still in the

pipet, orient it over the wound in the host. Insert the graft by gently blowing on the pipet. (If this fails, the graft tissue can be dislodged gently from the pipet with a broom straw or similar device.) Final fitting is accomplished by gentle pressure with a fine blunt-tipped glass rod.

3. One or more of the thin strips (1 mm thickness) of agar is placed over the graft to hold it in place. The strips should be 2-3 times as wide as the graft and 2-3 cm long. The strips will adhere to the agar surface of the dish.

4. The Petri dish is covered and stored in a cool (12-18°C), dark place.

The absence of water and light serve to restrict locomotor activity of the grafted animal, while the agar maintains a sufficiently high humidity. A high humidity can be ensured also by either of two means: either by making two or three 5 mm-diameter wells containing water in the agar, or by placing a wet piece of filter paper inside the top of the Petri dish. In cases where a perfect fit of the grafted tissue has been attained, it may be possible to dispense with the thin agar "bandages". Successful implantations will heal in-place in 24-36 hours, depending on the temperature. When the graft has taken, the Petri dish is flooded with tap water and the agar "bandages" are removed carefully.



**FLEXIBLE TUBING
TO MOUTHPIECE**

FIG. 1

Figure 13. The pipet used for implantation grafts.

Soft glass 7 mm tubing is drawn out to a capillary with the aid of a Bunsen burner flame. At the region of desired diameter, the capillary is bent by gentle heating. Obviously, the diameter will determine the size of the implant to be made. The pipet is trimmed even by scoring with a file or diamond pencil and breaking. For easy handling the overall length should be 5-6 inches and the pipet should be fitted with flexible tubing to a mouthpiece.

II. "Butt grafting" technique:

The principle of this technique is to immobilize the experimental animals by embedding them in agar, following by cutting both agar and worm and rearranging appropriately the cut embedded parts. The first critical part of the technique is to immobilize the worm perfectly in the horizontal plane; the second is to cut and rearrange as rapidly as possible.

The procedure is simple:

1. A worm is transferred, free of water, to an agar-lined Petri dish. Adhering water may be removed by blotting the agar with filter paper. Molten 2% agar at 43°C (close to the gelation point) is quickly poured over the animal and allowed to solidify. Gelling may be accelerated by having the Petri dish chilled and by a stream of air passing over the freshly poured agar. Grafting will be difficult or impossible if the worm is not extended and horizontal.

2. When the agar has solidified an oblong block of agar containing the worm is cut out with a razor blade. The block is transferred onto the agar surface of another Petri dish.

3. To illustrate the actual grafting process, the following description is for grafting the head onto the tail of the same planarian. This is diagrammed in Figure 14. A square block of agar, as wide as that containing the worm, is placed next to embedded animal. The block containing the planarian is cut so as to sever the tail and the head. The embedded head is transferred to the cut tail surface and the plain agar block is placed against the anterior cut. It is most important to have a snug fit and good alignment of the cut surfaces. To keep the agar blocks together, several thin agar strips are arranged in criss-cross fashion over the entire assemblage.

Aside from working rapidly, the technique is facilitated by keeping the worm as cool as possible ... by chilling the bottom layer of agar; by using a worm kept cool and/or lightly anesthetized, and by cooling the embedded worm prior to cutting. Healing time varies between 24 and 48 hours depending on the quality of the graft and the temperature. The grafted animals are removed from the agar by covering the block with water and splitting it apart with blunt glass needles.

III. Comments.

The two methods outlined here are capable of adaptation to suit many experimental designs. For example, the implantation technique has been used to graft radio-isotopically labelled parts into normal hosts in our radioautographic studies on regeneration. The "butt graft" can be used to join parts of two or more planarians into a chimeric association. Preliminary experiments with the latter technique have produced bipolar worms with the regenerated head dominant to the grafted head, as far as locomotor behavior was concerned. It may be possible to use either technique for grafting between different species of planarians.

✱ It is conceivable that these techniques can be combined with the superimposition of electrical (Marsh and Beams, 1952) or chemical gradients (Flickinger, 1959; Flickinger and Coward, 1962) and lead to further understanding of the problems in planarian biology.

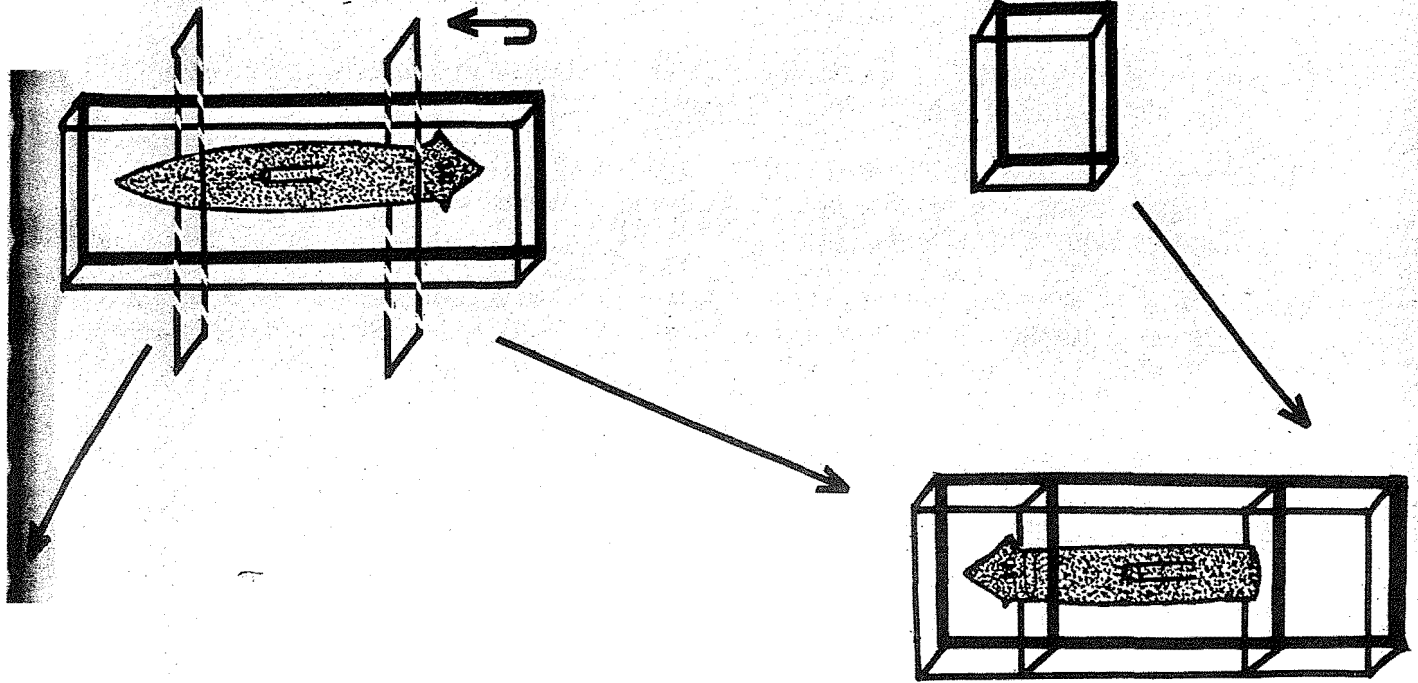


FIG. 2

Figure 14. Schematic representation of the "butt graft" technique for the example described in the text.

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Section V

THE EXTRACTION OF RIBONUCLEIC ACID FROM THE PLANARIAN, CURA FOREMANII

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Introduction

In this method for the extraction of RNA (Ribonucleic acid) a 2-phase phenol-aqueous buffer system is used. The phenol precipitates or dissolves protein and DNA (Deoxyribonucleic acid), while leaving the RNA in the aqueous phase. The extraction is carried out in the cold to minimize degradation of RNA. Although it is now common to perform the extraction hot with most tissues, this procedure was found to degrade planarian RNA.

Extraction

During the entire procedure, all materials were kept either in a refrigerator at 2°C or on ice.

1. The planarians were collected by cooling the home bowl, dislodging the animals from the walls with a soft brush and pouring them into a graduated centrifuge tube. The tube was spun at low speed to pack the animals, and their volume was read. A volume of 0.2 to 0.3 ml, corresponding to 100 to 150 medium-sized worms, was used in the extraction. The worms were transferred with a small amount of water to a 3 ml conical glass homogenizer, and the water was carefully discarded.

2. The animals were homogenized with 3.0 ml of buffer, containing 0.01 Molar sodium acetate (pH 5.1), 0.1% sodium lauryl sulfate (a detergent) and 10 ug/ml bentonite (a powerful inhibitor of ribonuclease).

3. The homogenate was transferred with 1.0 ml buffer to a 10 ml beaker which contained 4.0 ml water-saturated phenol (about 12% water). The mixture was stirred vigorously with a magnet for 1 hour, then transferred to a 10 ml polypropylene centrifuge tube.

4. The tube was spun at 2°C for 4 minutes at 20,000 x gravity.

5. After being spun, the tube contained 3 layers: a bottom, dark layer of phenol; a middle, small, white layer of precipitated protein; and a top aqueous layer. The phenol layer was drawn from the tube by inserting a 4 ml volumetric pipette through the aqueous and protein layers, blowing out any material from these layers that may have lodged in the tip, then aspirating and discarding the phenol.

6. The aqueous phase and residue were treated as the homogenate in step 3. Stirring was for 1/2 hour.

7. As no. 4, with an 8 minute spin.

8. The aqueous layer was carefully transferred to a 15 ml graduated conical centrifuge tube. The phenol and residue were discarded. If the aqueous was not clear, the tube

was spun for 5 minutes in a table top centrifuge, and the clear supernatant was decanted into another centrifuge tube.

9. 1/10 volume 20% potassium acetate and 2.5 volume 95% ethanol were added to the tube with stirring. The precipitate was spun down in a table top centrifuge for 5 minutes.

10. The precipitate was washed once with 4 ml ethanol, water (3:1), then redissolved in a volume of distilled water equal to the aqueous volume in step 8. Step 9 was repeated.

11. If the precipitated RNA was dissolved in water for injection into planarians, traces of ethanol were removed by washing the solution with ether a few times and blowing the ether off the chilled solution with nitrogen or air.

Remarks

This method was described for a convenient small quantity of planarians. If another quantity was used, the volumes of buffer and phenol in steps 2 and 6 were adjusted proportionately. If the volumes were too low, a gel was formed and mixing was poor; if too high, RNA did not precipitate in step 9.

If a high speed centrifuge is not available for step 4, the tube can be spun at low speed, but the time of centrifugation must be increased proportionately. The product of gravities and minutes should be around 80,000.

Purity and Yield

The concentration of RNA in solution is easily determined by a spectrophotometer. With a wavelength of 260 millimicra and a cell light path length of 1 cm, 100 ug/ml RNA gives an optical density of 2.0.

From this relation of optical density and concentration, the purity of the extracted RNA was found to be less than 50%. The nature of the impurity was not determined, but it is probably largely polysaccharide. Black pigment in the RNA is noticeable by eye (especially in the heavily pigmented species, Cura foremanii), but it probably is not much of a percentage by weight of the product. Protein could not be detected. A number of attempts to purify the RNA were made, by methods including chromatography and electrophoresis, but all without success.

The yield, measured spectrophotometrically, was 3.5 mg RNA/ml worms or 6 ug RNA/worm. The weight of a single worm, blotted dry, was found by a separate weighing to be in the range 1-3 mg. Assuming 2 mg as an average weight, the yield is 3 mg RNA/g worms, a figure that compares with the values found for many other kinds of tissue.

Section VI

AN APPARATUS FOR INJECTING PLANARIANS^{1,2}

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Thompson and McConnell (1955) reported the occurrence of classical conditioning in the planarian, *Dugesia dorotocephala*, using light as the CS, shock as the US, and contraction or cephalic turning as the CR. A number of subsequent studies have investigated learning and retention in planarians; these studies have been reviewed by Jacobson (1963). Recently, McConnell (1962) studied the conditioning process in cannibal planarians that had ingested the tissue of other planarians. He found that planarians that cannibalized conditioned planarians were initially more responsive to the CS than planarians that cannibalized unconditioned planarians. This finding suggested further experiments in which planarians are tested after being injected with various tissue extracts from conditioned planarians. In experiments of this sort, however, planarians tend either to be injured by the injection process, or else to extrude the injected substance within a few minutes. The purpose of the present paper is to describe an effective, inexpensive procedure for injecting planarians with approximately known quantities of a solution.

The function of the apparatus (Fig. 15) is to hold a syringe in a fixed vertical position, and to provide manipulation of the syringe in the vertical dimension with reasonable precision. The apparatus is made from a ring stand, three lengths of metal rod, three double C clamps, a sleeved C clamp, a standard test-tube clamp, a spring, and a threaded bolt surmounted by a knurled knob. Two double C clamps hold two rods (R1 and R2) parallel to each other and perpendicular to the ring stand. Another double C clamp is used to attach the remaining rod (R3) perpendicularly to the upper of the two parallel rods (R2), in a nearly horizontal position. The sleeved C clamp fits over R1 and forms a hinge. This sleeved C clamp holds the test-tube clamp perpendicular to R1. The spring is permanently attached to R3 and the test-tube clamp, and holds them roughly parallel. A threaded rod (40 turns/in.) is threaded through R3; its rounded lower end rests in a small depression milled into the arm of the test-tube clamp. The syringe (Hamilton 50 μ l.) is held firmly in the jaws of the test-tube clamp, and may be moved up and down with considerable sensitivity by turning the knob surmounting the threaded rod. The lengths of R3 and the test-tube clamp (about 6 in.) ensure that the arc described by the syringe does not differ appreciably from a straight line, over a short distance.

A glass micropipette, drawn from capillary tubing, is glued to the inside of an 18 gauge hypodermic needle and extends from the end of the needle, which in turn fits on the syringe. Directly below the needle, a solid brass cylinder stands firmly in the center of a bowl filled with ice. A planarian is placed, dorsal side down, on a sheet of aluminum foil on the brass cylinder, and the position of the bowl is adjusted so that the needle, when lowered, will strike the planarian about halfway between the head and the pharynx (Fig. 16³). The scene is strongly illuminated, and a microscope is focused on the planarian. The needle is slowly lowered to puncture the planarian, and is then retracted very slightly to avoid plugging the tip. Using gentle pressure on the plunger, the planarian is injected with a solution. If a brightly colored solution is used, the color is observed to disperse immediately throughout the planarian's body.

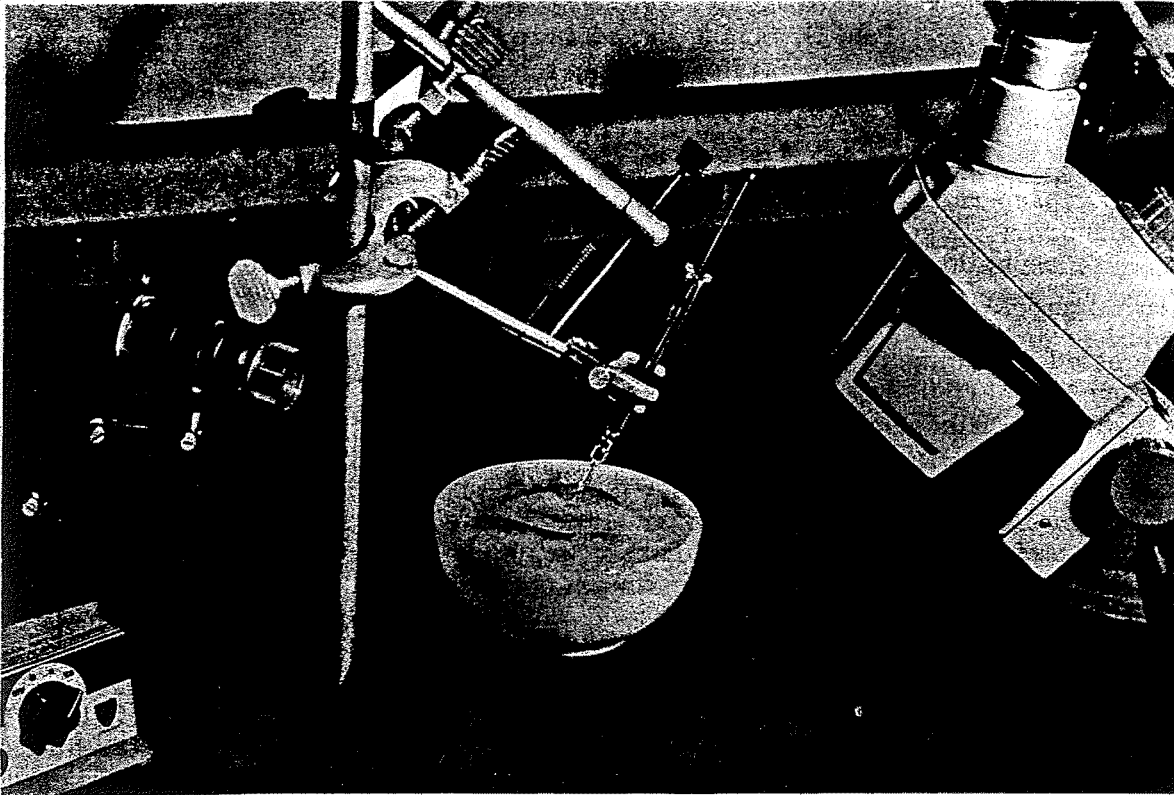


Fig. 15. Injection apparatus.

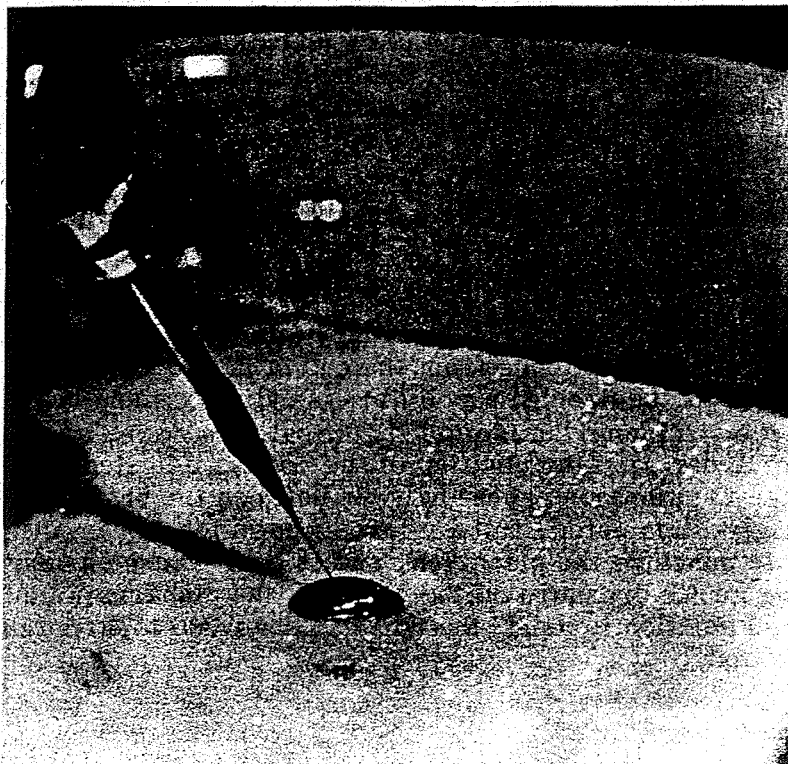


Fig. 16. A planarian being injected.

To study the effectiveness of the procedure, 12 planarians (*D. dorotocephala*, about 2 cm. in length) were each injected with 2 μ l. of a solution of ribonucleic acid (RNA) extracted from planarian tissue homogenate. Three other planarians served as sham injectees: they received identical treatment including puncture, but were not injected, and were returned to the same bowl as the injectees. To a small quantity of the solution in which the planarians are normally maintained (.4 mg./ml. NaCl, .07 mg./ml. CaCl₂, and .01 mg./ml. NaHCO₃; pH = 6.5), a vital dye (eosin Y) was added to give a concentration of 4.0 mg./ml. The pH, raised to 8.0 by the dye, was adjusted to 6.5 with HCl. Planarian RNA was then added to give a concentration of 3.0 mg./ml. Of the 12 planarians that were injected with this solution, 9 received but one puncture and remained intact, while 3 received multiple punctures and were discarded.⁴ The 9 remaining planarians each extruded an indeterminate amount of the colored solution within a few minutes after being injected, but were nevertheless readily distinguishable by their color, one day later, from the sham injectees. Each injectee initially received 6 μ g. of RNA (approximately the amount ordinarily present in one planarian), so that each probably retained as much RNA as if it had cannibalized at least half of another planarian. One injectee died; in the other 8, no ill effects from the injection procedure were apparent after three days.

References

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- McConnell, J. V. Memory transfer through cannibalism in planarians. J. Neuropsychiat., 1962, 3, suppl. 1, S42-S48.
- Thompson, R., & McConnell, J. V. Classical conditioning in the planarian, Dugesia dorotocephala. J. comp. physiol. Psychol., 1955, 48, 65-68.

Footnotes

1. This paper is reproduced, with permission, from Perceptual and Motor Skills, in press.
2. Apparatus development was supported by AEC Contract AT(11-1)-825 and NIMH Grant MH 02946-05S1 to J. V. McConnell. Work was carried out during the first author's tenure as an NIH Postdoctoral Fellow (Fellowship No. 1-F2-MH-22, 490-01); the second author held a Dr. Louis Gelston Fellowship from the University of Michigan Medical School.
3. Acknowledgement is made to H. T. Salive, who photographed the apparatus.
4. In these animals, the needle penetrated both the dorsal and the ventral surfaces and the solution was injected chiefly outside rather than inside the animal. Once an animal has received multiple punctures, it will not readily retain an injected solution.

Section VII

AN ANNOTATED BIBLIOGRAPHY OF RESEARCH ON PLANARIANS

Compiled by

Margaret L. Clay and Ursula Mosler

(One of the most important personality traits a good scientist can possess is an enthusiasm to get into the laboratory and get things done. Without this necessary urge on the scientist's part, few experiments would ever be undertaken and fewer still ever finished. But no behavioral scientist can undertake adequate research without having at least a minimal amount of background knowledge about the animals with which he will be working and without knowing a great deal about the sorts of results that other experimenters have gotten in the past when working with the same organism. To many young scientists, I fear, the very idea of library work, of boning up on "past history," is distressing. Unfortunately, library work simply cannot be avoided, and that's that. Scientific journals are not usually found in high school libraries, however, and it may take some ingenuity for the beginning behaviorist to obtain access to many of the more esoteric or unusual periodicals. The wise scientist learns the following rule early in life, though: take all such problems to the nearest librarian! That's what librarians are there for, and if your own library doesn't have the book or journal that you need, perhaps a loan can be arranged. Then, too, most universities and colleges will give high school students access to their books and journals if the student is sponsored by one of his teachers or by the high school librarian. Whatever the case, the young scientist can save himself a tremendous amount of time and effort by becoming thoroughly grounded in "the literature" before he begins his own experimental work.

(For a number of years now, the WORM RUNNER'S DIGEST has published in each issue an "Annotated Bibliography of Research on Planarians," covering not only the fields of behavior and regeneration, but genetics, morphology, physiology, etc. This continuing bibliography is perhaps the most complete and up-to-date of any such in the world, particularly as far as behavioral studies are concerned. Most of the important references the starting psychologist will need are described in the following 42 pages of the MANUAL; those articles that appeared in the WORM RUNNER'S DIGEST are listed separately, but are not annotated.

(As far as general references are concerned, Hyman's many writings on the flat-worms are classic, although a little out of date at the present time. A student interested in regeneration should read the writings of Brondsted and of Wolfe perhaps above all others. And Allan L. Jacobson's survey of the various experiments involving learning in planarians is a "must" for any one planning to undertake behavioral studies.

(Good Reading! ---James V. McConnell)

REVIEWS AND GENERAL INFORMATION

1. Hyman, L. H. The Invertebrates. Vol. 2: Platyhelminthes and Rhynchocoela. New York: McGraw-Hill, 1951.

An authoritative and fairly comprehensive coverage of all aspects of flatworms through 1949. Considered the best single source on this subject.

2. Hyman, L. H. Order Tricladida, in Ward, H. B. and Whipple, G. C. (eds.) Fresh Water Biology. New York: Wiley, 1959. Chapter 13, pp. 326-334.

A recent and brief taxonomic key to the identification of planarian species with helpful diagrams. This article also includes references to more detailed taxonomic and ecological studies.

3. Kenk, R., The fresh-water triclads of Michigan. Miscell. Publ. Mus., Zool., The University of Michigan, 1944, no. 6.

Recognition of a planarian species is often made difficult by duplicate names. Thus, Dugesia tigrina is identical to Dugesia maculata. Kenk, in this article, aids the layman by grouping together alternative names for the same species. He also provides detailed descriptions of the external features, anatomy, taxonomy, ecology and distribution of the various species.

4. Jennings, J. B. Observations on the nutrition of the land planarian Orthodemus terrestris (O. F. Muller) Biol. Bull., 1959, 117, no. 1, 119-124.

(1) The land planarian Orthodemus terrestris feeds principally upon small slugs and earthworms which are captured after chance encounter.

(2) The typical triclad method of feeding, with the protruded cylindrical plicate pharynx inserted into the prey to disrupt and withdraw the body contents, is used without modification.

(3) Disintegration of the food during ingestion is so effective that the resultant particles are available for immediate phagocytosis by the gut cells and intraluminal digestion is absent.

(4) The food reserves consist of fat stored in the mesenchyme and columnar gut cells, and protein stored in gastrodermal "sphere-cells." Protein reserves are depleted during the breeding season and replenished in the late summer and autumn.

(5) It would appear that the basic triclad methods of feeding and digestive processes are quite adequate to the needs of terrestrial life and Orthodemus shows no particular adaptation to this so far as nutrition is concerned.

LEARNING AND OTHER BEHAVIOR

1. Jacobson, A. L. Learning in flatworms and annelids. Psychol. Bull., 1963, 60, 74-94.

This article attempts an exhaustive review of the research purporting to demonstrate behavioral modifications in earthworms, planarians, and related organisms. Studies are grouped first according to phylum, and for each of the phyla considered according to certain subcategories of learning: habituation, classical conditioning,

instrumental learning, and variability. Examination of the literature reveals that whereas earlier work was often ill-controlled, more recent research has for the most part been rigorous and convincing. It is concluded that learning and related phenomena have indeed been demonstrated clearly in each of these two phyla, and that research on these animals provides a promising means of investigating the "molecular" basis of learning.

2. Best, Jay Boyd, *Protopsychoogy*. Sci. Am., 1963, 208, 55-64.

A review of planarian research from McConnell and Thompson to date, with emphasis on the relevance of this research to whether the vertebrate brain is necessary or only sufficient to produce motivated, flexible behavior. Best's own research with flatworms in mazes is discussed at length, and detailed pictures are included of the apparatus and procedure used. He presents evidence that "psychological" behavior patterns seen in vertebrate forms during learning are replicable with planarians (i. e. lethargy, anxiety and conflict behavior). Best suggests that there are protopsychological properties at a cellular or subcellular level of organization within even primitive animal forms. Better understanding of these, he contends, will shed new light on the ultimate structure of the human brain and the true role of nerve circuitry in this structure.

3. Hovey, H. B. Associative hysteresis in marine flatworms. Physiol. Zool. 1929, 2, 322-333.

Associative hysteresis has been demonstrated by the conditioned reflex method in the flatworm Leptoplana. The animal is photokinetic. Through simultaneously exposing the worm to light and preventing it from creeping forward, an inhibition of photokinesis was effected. Light was the biological stimulus for movement, and contact was the biological stimulus for prevention of movement. Light became also a conditioned stimulus for prevention of movement. Thus light was conditioned to inhibit a response which it itself elicited. Experiments with worms whose cephalic ganglia had been extirpated indicated that these organs took part in the learning.

4. Thompson, R. & McConnell, J. V. Classical conditioning in the planarian, Dugesia dorotocephala. J. comp. physiol. Psychol., 1955, 48, 65-68.

The purpose of the present experiment was to study the facility with which planarians (D. dorotocephala) form CR's in a classical light-shock conditioning situation. Forty animals were employed. They were divided into four equal groups: the experimental group and three different control groups.

It was found that for the experimental group there was a significant rise in the number of contractions and turning responses as a function of the number of trials. That these increments in frequency of responses were not caused by sensitization to either shock or light stimulation was indicated by the performance of the control groups.

5. Halas, E. S., James, R. L., & Knutson, C. An attempt at classical conditioning in the planarian. J. comp. physiol. Psychol., 1962, 55, 969-971.

The purpose of the present experiment was to reproduce the results of the Thompson and McConnell (1955) study, which indicated that classical conditioning could be established in planarians. The present experiment divided 48 planarians into an experimental group, a light control group, a shock control group, and a normal response control group. The results agree in general with Thompson and McConnell's, but it is suggested that reflex sensitization is a more tenable interpretation of the data.

6. Halas, E.S., Mulry, R.C., & Deboer, M. Some problems involved in conditioning planaria: electrical polarity. Psych. Reports, 1962, 11, 395-98.

Twenty planarians were randomly divided into two groups and then trained in a narrow trough to approach a non-preferred light. Electrodes were mounted at each end of the trough. For Group I, the electrode at the right end was always negative whereas, for Group II, the electrode at the right end was always positive. Planarians were found to be more sensitive to an electrical current when they were approaching the anode as compared to when they were approaching the cathode. Sensitivity was measured by the number of electrical shocks and time required to induce the planarians to reverse their direction of travel. The findings suggest that one or more variables are not properly controlled in studies attempting to condition planarians.

7. McConnell, J.V., Jacobson, A.L., & Kimble, D.P. The effects of regeneration upon retention of a conditioned response in the planarian. J. comp. physiol. Psychol., 1959, 52, 1-5.

The purpose of the present experiment was to study the effects of regeneration upon retention of a conditioned response in the planarian (D. dorotocephala). A classical conditioning situation was employed in which light was the CS and shock the US. Fifteen animals were divided into three equal groups, an experimental group and two control groups. The experimental animals were first conditioned to a criterion of 23 responses in 25 consecutive trials. They were then cut in half transversely and allowed to regenerate. Following regeneration, both head and tail sections were retained to the original criterion.

It was found that both head and tail sections showed significant retention of the CR and to the same degree. That these findings were not the result of sensitization due to the processes of cutting and regeneration was indicated by the performance of one of the control groups. That the small amount of "forgetting" of the CR which occurred in the experimental animals was probably due merely to the passage of time was indicated by the performance of the other control group.

It was concluded that in planarians the rudimentary brain is necessary for learning to take place but not for retention of the learned response. Two intriguing questions posed by these findings are: How does the tail retain anything? and When the tail section regenerates a new brain, is the CR "built into" the cerebral ganglia?

8. McConnell, J.V., Jacobson, Reeva, & Maynard, D.M. Apparent retention of a conditioned response following total regeneration in the planarian. Amer. Psychologist, 1959, 14, 410. (Abstract)

Data reported in this paper suggest that significant savings of light-shock conditioning occurs even in "third generation" animals composed entirely of reformed tissue, although the savings is not as great as that found in "second generation" animals.

9. McConnell, J.V., Cornwell, P.R., & Clay, Margaret. An apparatus for conditioning planaria. Amer. J. Psychol. 1960, 73, 618-622.

This paper presents a modification of an apparatus used in conditioning planarians (with light-shock pairing) that is more efficient and more economical than the one used in earlier studies.

10. Westerman, R. A. Somatic inheritance of habituation of responses to light in planarians. Science, 1963, 140, 676-677.

Planarians show long-lasting reversible decrease of responses to a photic stimulus repeated 25 times each day. This habituation was found to be significantly faster in regenerated offspring of previously habituated planarians, and was also transferred by cannibalization, thus resembling light-shock conditioning. Habituation provides a new situation in which to study the somatic inheritance of learning in planarians.

11. Barnes, C. D. & Katzung, B. G. Stimulus polarity and conditioning in planaria. Science, 1963, 141, 728-730.

Orientation in the monopolar pulse field used as the unconditioned stimulus was found to influence formation of a conditioned response to light in planarians. Planarians trained while oriented with the head toward the cathode reached maximal response rates rapidly, while those trained while oriented toward the anode showed no evidence of conditioned response formation.

12. Baxter, R. & Kimmel, H. D. Conditioning and extinction in the planarian. Amer. J. Psych., 1963, LXXVI, no. 4.

This study compared paired and unpaired CS-UCS in the planarian at two intensive levels each of the CS and UCS. Twenty-four animals were divided into the eight subgroups of the 2 x 2 x 2 factorial design. The CS was either a weak or strong light of 3-sec. duration and the UCS was either a weak or strong electric shock of 1-sec. duration. The Ss in the paired conditions received the UCS during the last 1 sec. of the CS, while those in the unpaired conditions received the stimuli at least 15 sec. apart. Each S had 50 trials of paired or unpaired CS-UCS per day for 5 days and 50 extinction trials on the sixth day. The dependent variable was the number of CRs, a CR being a longitudinal contraction of the animal's body which occurred within 2 sec. after the onset of the CS.

The curves showing the frequency of the responses of the paired and unpaired groups diverged increasingly throughout conditioning. This difference was statistically significant as were those which favored the strong CS over the weak CS and the strong UCS over the weak UCS. None of the interactions was significant. The difference between the paired and unpaired groups which was observed during conditioning vanished, however, during the 50 extinction-trials. The difference due to the intensity of the CS and UCS were still present during extinction, although the former difference was largely confined to the unpaired group.

It was concluded that the difference in the frequency of the responses during conditioning between the paired and unpaired groups was due to the contiguous CS-UCS relationship which obtained during conditioning for the paired group but not for the unpaired group. Whether this difference could be taken as evidence for 'true' conditioning rather than alpha conditioning in the planarian could not be resolved, since the effect vanished as soon as the UCS was removed during extinction and it has been shown that light alone is capable of producing longitudinal contractions in this organism. The effects of the intensity of the CS and UCS did not interact with the CS-UCS contiguity-variable and were, therefore, assumed to have influenced performance rather than conditioning.

13. Condaró, L. & Ison, J. R. Psychology of the scientist: X. Observer bias in classical conditioning of the planarian. Psychol. Rep., 1963, 13, 787-789.

An experiment purporting to show the susceptibility of planarian research to

observer bias.

14. Shinkman, P. G. & Kornblith, Carol. Comment on observer bias in classical conditioning of the planarian. Psychol. Rep., 1965, 16, 56.

Reviews Condarò and Ison's study and indicates why the experimental conditions they used may be inappropriate and insufficient for the conclusions they drew.

15. Crawford, F. T., Livingston, P. A. & King, F. J. The effect of massed and spaced trials upon the classical conditioning of planaria. Am. Psych., 1964, 19, 485 (Abstract).

80 Ss were divided into 4 groups receiving training conditions of either light, shock, light paired with shock, or neither light nor shock. Half of each of the groups were given trials 30 sec. apart and the remainder were given trials 60 sec. apart. Conditioning occurred only among Ss given paired light and shock, with spaced training producing significantly greater conditioning. Half of all Ss were then given either massed or spaced extinction trials. Massing and spacing of acquisition trials were found to be of more significant influence upon rate of extinction than were the massing and spacing of extinction trials.

16. VanDeventer, J. M. & Ratner, S. C. Variables affecting the frequency of response of planaria to light. J. comp. physiol. Psych., 1964, 57, 407-411.

The results of Experiment 1, designed as a replication of the retention-following-regeneration study by McConnell, Jacobson and Kimble (1959), indicate that a Regeneration Control group that had not been trained prior to cutting and regeneration performed as well on training trials as an Experimental Group that had been trained prior to and after cutting and regeneration. These results are in contrast to prior findings by McConnell et al. in which the Regeneration Control group did not show this sensitization following regeneration. In the present study, the animals were kept in the dark during the several weeks required for regeneration to take place; in the prior study, the animals were exposed to light daily. Thus it is possible that both Experimental and Control animals in the present study became over-sensitized to light during the regeneration period, and that this sensitization masked any "savings following regeneration" that might have obtained.

Experiments 2 through 6 indicate that a number of variables, uncontrolled for in Experiment 1 and in some other similar experiments, affect responsiveness of planarians to light. Specifically, the results of these experiments indicate: (a) Responsiveness to light increases as a function of temperature and dark-adaptation, and that the shape of the training trough is important in the species D. tigrina; (b) Responsiveness to light varies inversely with the size of the animal for the species C. foremanii; (c) A change from creek to tap water, "starvation," and environmental contamination (of a sort) do not affect responsiveness to light for D. tigrina.

17. Griffard, C. D. Classical conditioning of the planarian Phagocata gracilis to water flow. J. Comp. physiol. Psych., 1963, 56, 597-600.

In an attempt to determine whether or not planarians can be conditioned, 40 planarians, P. gracilis, were studied. The experimental group received 250 pairings of water flow (CS) and shock (US). A shock control group received 250 exposures to the US and a flow control group received 250 exposures to the CS. A 3rd control group

received no training but was tested in the same manner as the other groups. All Ss of a specific group were given 25 test exposures to the CS from 10-15 hr. after training. The difference in the performance of the experimental group as compared with all other groups was significant at the .005 level. Additional evidence indicated that some sensitization may have occurred in the shock control group. The extent to which sensitization influenced the results for the experimental group is uncertain and therefore an interpretation in favor of classical conditioning is considered equivocal.

18. Griffard, C.D. & Peirce, J. T. Conditioned discrimination in the planarian. Science, 1964, 144, 1472-3.

To demonstrate classical conditioning in the planarian in a situation uncontaminated by the possible artifacts of pseudoconditioning or sensitization 30 Phagocata gracilis were successfully trained, by the use of directional shock as the unconditioned stimulus, to turn in one direction to a light, and in the opposite direction to vibration. Ten similarly trained planarians tested by an independent observer who did not know the previous training conditions experiences by any animal showed similar results.

19. Corning, W. C. Evidence of right-left discrimination in planarians. J. Psych., 1964, 58, 131-139.

The ability of planarians to perform a right-left discrimination has been examined. It was observed that 10 of 17 trained subjects were able to achieve a criterion of nine out of 10 correct responses, while none of the 16 control animals succeeded in attaining the same level of performance. However, the subjects of both the trained and the control groups displayed a marked tendency to shift their turning preference, suggesting that some factor peculiar to the maze situation prevents response stabilization.

20. Van Oye, P. Over het geheugen bij de flatwormen en andere biologische waarnemingen bij deze dieren. Natuurwet. Tijdschr., 1920, 2, 1-9.

Planarians learned to obtain food by descending a wire placed in the water of the animal's bowl. The response was acquired only if a gradual training regimen (resembling operant "shaping") was employed; was maintained in a series of tests at 2-day intervals; and disappeared after a lapse of 2 months.

21. Lee, R. M. Conditioning of a free operant response in planaria. Science, 1963, 139, 1048-1049.

The response of breaking a photoelectric cell beam was automatically recorded and reinforced. Termination of an intense light was the reinforcement in an escape conditioning situation. The rate of response for the experimental subjects was significantly different from that of controls matched for equivalent changes in light intensity.

22. Corning, W. C. & John, E. R. Effects of ribonuclease on retention of conditioned response in regenerated planarians. Science, 1961, 134, 1363-1365.

Conditioned planarians were transected and allowed to regenerate in a ribonuclease solution or in pond water. Heads which had regenerated in ribonuclease displayed a retention level equal to that of head and tail sections which had regenerated in pond water. However, tails regenerated in ribonuclease performed randomly although they could be retrained to criterion.

23. Best, J. B., & Rubinstein, I. Maze learning and associated behavior in planaria. *J. comp. physiol. Psychol.*, 1962, 55, 560-566.

Planarians were trained in simple two-choice delta and Y mazes in which removal of water was the incentive to "run" the maze, its restoration the reinforcement for a correct choice, and light and darkness the cues. Some were reinforced on the lighted and some on the darkened alternative. Both exhibited an initial phase of enhanced preference for the reinforced alternative, i. e., learning, followed by active rejection of the reinforced alternative. These results are not derived from sensitization or desensitization to light or water, nor from accrual of substances in the water, nor from spoors of previous passages. The decrease in correct choices was followed by a lethargic state which was not the result of fatigue or injury. Phenomena resembling vicarious trial and error and conflict behavior were observed.

Seven Ss were subsequently tested in either a rim maze or dual maze, modifications of the Y maze. No rejection of the reinforced alternative or lethargy was observed in the rim maze, which made a larger chamber available to S between trials. In the dual-maze situation, one S learned to choose the lighted alternative in a Y maze with rough well walls and the dark alternative in one with smooth well walls, and showed neither rejection of the reinforced alternative nor lethargy.

24. Jacobson, A. L. An attempt to demonstrate transfer of a maze habit by ingestion in planaria. 1962, University of Michigan, dissertation.

Previous experiments have demonstrated that transfer of a classically conditioned response can occur in planarians by means of ingestion of the tissue of trained planarians. In these experiments, the learning involved was an association between light (CS) and shock (UCS), and the response was a conspicuous muscular response. The present experiment attempted to determine whether this same phenomenon, transfer by ingestion, occurs also in a maze-learning situation.

Planarians were trained to turn to either the right or left side in a single-unit, undifferentiated T-maze. After an initial preference assessment, each worm was trained to its own non-preferred side. Upon choosing correctly, an animal was immersed in its home bowl. If it chose incorrectly, the animal was required to run the maze again, and again if necessary, until it had chosen correctly. After three consecutive incorrect runs on a given trial, the animal was forced to the correct side and immersed in the home bowl. In addition, an incorrect choice resulted in the animal's being exposed to a brief burst of bright light.

When a given animal had satisfied a criterion of 9 correct choices out of any 10 consecutive trials, its training was terminated for a period of one week. At the end of this period, retraining was instituted, and was continued until the animal attained the same criterion a second time. Thereupon, that animal was cut into three pieces, and each piece was fed to an untrained cannibal worm. Concurrently, an untrained worm was cut and fed to other untrained cannibals. This feeding process was repeated at several day intervals until each cannibal, experimental or control, had eaten trained or untrained worms, respectively, a total of three times. Training was then begun for the cannibal worms. All training was conducted "blind", i. e., in such a fashion that E did not know the group membership or past performance of any of the worms.

In terms of number of trials to criterion, or number of initial correct responses, the hypothesized difference between the groups did not occur. This failure does not lead,

however, to a complete rejection of the original hypothesis. Data on retraining indicated that the habit established in the present experiment was an unstable one. It was thought reasonable to assume that transfer of a T-maze habit might well occur if a more durable habit were employed. Since classical conditioning in these animals is remarkably persistent, it is likely that a habit of comparable stability can be established in the T-maze situation. Suggestions were made as to how the present learning situation might be changed to prolong the performance of the learned response.

Consideration was also given to other factors which might have been responsible for the failure to find differences between the groups.

25. McConnell, J. V. Memory transfer through cannibalism in planarians. J. Neuropsychiat., 1962, 3 (suppl. 1), 542-548.

Planarians are capable of learning a simple conditioned reaction. This learning survives cutting and regeneration, and the memory storage mechanism has a biochemical component, probably ribonucleic acid, which is distributed throughout the body. Learning seems to be transferrable via cannibalistic ingestion.

26. Hartry, Arlene L., Keith-Lee, Patricia, & Morton, W.D. Planaria: Memory transfer through cannibalism reexamined. Science, 1964, 146, 274-5.

Planarians which have cannibalized untrained planarians exposed to photic stimuli only, handling only, or previous conditioning, all require significantly fewer trials in a conditioning situation than naive planarians.

It should be noted that this is the only cannibalism study so far published in which the animals were given massed conditioning trials (approximately 150 trials all in one session) rather than spaced trials. Whether this difference "makes a difference" has not as yet been determined.

27. McConnell, J. V. Cannibalism and memory in flatworms. New Scientist, 1964, 21, 465-8.

The author's experiments during the last few years, which have suggested the existence in planarian worms of a chemical memory distributed through the body, have aroused wide interest. Here, he explains and discusses the experiments and reflects on their implications for learning theory.

28. Bennett, E. L. & Calvin, Melvin. Failure to train planarians reliably. Bulletin (Neurosciences Research Program), July-August, 1964.

A limited distribution article describing unsuccessful efforts over a two year period to train planarians to produce a reliable, stable response which might be used in later biochemical studies of memory.

29. McConnell, J. V. Failure to interpret planarian data correctly: A reply to Bennett and Calvin. (Available on request from the author % Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan)

A critique of Bennett and Calvin's limited distribution paper which accepts their data but questions their interpretations of the data. Available by request only.

30. Landauer, T. K. Two hypotheses concerning the biochemical basis of memory. Psych. Review, 1964, 71, 167-179.

Two hypotheses concerning a possible molecular basis of memory have been offered. The first is that the basic event of conditioning or learning is the transfer of RNA molecules from surrounding glial cells to conducting neurons and subsequent transformation of the protein synthesizing apparatus of the neuron. The second is that information is coded for storage in the CNS in the form of frequency characteristics of spreading action potentials to which the membranes of neurons can be tuned.

The notion of a chemical interaction between glia and neurons is based in part on the recent findings of Hydén (Hydén, 1961; Hydén & Egyhazi, 1962, 1963), and is at least vaguely in line with theoretical suggestions of Galambos (1961), while the idea of coding is reminiscent of the theory of "modulation" and "resonance" once proposed by Weiss (1952) to account for developmental innervation. The suggested mechanism by which transfer of RNA occurs selectively to active (US-UR) neurons is electrophoretic migration in the potential gradient of the action potential. Transformation of the incorporating neuron by the RNA is assumed to be somewhat analogous to that in viral or bacteriophage infection (Williams, 1959). Selective tuning is suggested as possibly based on the electrical reactance and rectifying properties of the membrane as described by Cole (1941). No mechanism is suggested for the specification of the RNA type to be transferred. The lack of such a specification mechanism would, of course, represent a critical flaw in the theory, even in the event that all the other postulated mechanisms were correct. Principally to make this lack clear, the proposal has been stated in terms of two separate and "freestanding" hypotheses, instead of in the guise of a unified theory.

It is obvious that these proposals are highly speculative. There is no direct evidence that the brain uses the mechanisms suggested, and the indirect evidence is consistent rather than compelling. However, some of the ideas are quite different from current conceptions of brain mechanisms in learning, and it is hoped, therefore, that they will be of some interest.

31. McConnell, J. V. Memories, molecules and minds. Sandorama (Sandoz Pharmaceuticals Publication), March, 1965.

A review and summation of information to date on research in planarian behavior and its implications for the role of RNA in biochemical memory.

32. Bardeen, C. R. The function of the brain in planaria maculata. Amer. J. Physiol. 1901, 5, 175-179.

(1) In Planaria maculata nerve cords and brain constitute the central nervous system.

(2) A fragment of this planarian will exhibit no sensory-motor coordination unless it contains a part of the central nervous system.

(3) If the anterior extremity of the body be removed by a transverse section passing through the eyes or through the body posterior to this region the worm will lose the power of recognizing food and of reacting normally towards it. The simple swallowing reflexes are maintained.

(4) For the more complex reactions of the individual the brain must be intact as far forward as the anterior margin of the eyes.

33. Pearl, R. The movements and reactions of fresh-water planarians. J. Micro. Sci., 1903, 46, 509-714.

The movements and reactions to all the more important stimuli, with the exception of light and heat, have been described and analyzed into their component factors in the body of the paper. A classic.

34. Mast, S. O. Reactions to temperature-changes in spirillum, hydra, and fresh-water planarians. Amer. J. physiol., 1903, 10, 165-190.

Planarians respond to weak and strong thermal stimuli, just as they do to weak and strong mechanical stimuli. That is, they turn towards the source of a unilateral stimulus applied to the anterior portion of the body, if it is weak, and away from it, if strong, and they respond by crawling if the stimulus is applied to the posterior portion of the body.

By gradually increasing thermal stimuli, applied equally on the sides and ends of the body, planarians can be made to give all the different reactions given in response to mechanical, chemical, rheotactic, and thigmotactic stimuli of different strength and applied to different regions of the body.

The reactions of planarians to thermal stimuli, depend, primarily, upon the intensity of the stimulus, and secondarily, upon the physiological condition of the animal and the location of the stimulus.

The nature of their reactions to stimuli in general bears little if any relation to the quality of the stimulus.

Planarians in their reactions do not orient themselves with reference to thermal rays of radiation or convection, i. e., the path which they follow is liable to form any angle with such rays.

Their reactions are due, apparently, to a general physiological change in the organisms, rather than to a direct effect on the motor organs or a mere simple motor reflex.

Of all the reactions induced by thermal stimuli, the negative reaction appears to be the only one that is under the conditions of the experiments directly protective or useful.

The optimum temperature, when the temperature is slowly increased, is 42°, and the minimum below 0°.

Planarians at ordinary temperatures react to an increase in temperature of from 2° to 3°.

35. Olmsted, J. M. D. Geotropism in Planaria maculata. J. animal Behav., 1917, 7, pp. 81-6.

(1) Unfed Planaria maculata which have been in the light are positively geotropic when first placed in the dark. After several days in the dark they become indifferent to gravity.

(2) Fed Planaria maculata which have been kept in the light are likewise positively geotropic at first. But they become negative after two days and indifferent after five days.

(3) Fed planarians which have been in the dark for some time are negatively geotropic.

(4) The presence or absence of slime tracks has no influence on the geotropism of these planarians.

36. Sun, K. H., & Dun, F. T. Note on geotropism in the dark-adapted Planaria gonocephala. Chin. J. Psych., 1936, I, pp. 1-9.

(1) When there is no change of the water for a week the majority of the unfed and dark-adapted Planaria gonocephala collect in the neck region of the flask.

(2) When the effect of the foul water is eliminated the majority of them remain in the bottom.

(3) The continuously fed worms which have been kept in the dark for seven days show indifferent distribution.

(4) In the light of our results we maintain that the so-called negative geotropism in the Planaria maculata as found by Olmsted is in all probability a chemically-induced phenomenon and is therefore not a "true response to gravity".

37. Brown, F. A. Responses of the planarian, Dugesia, and the protozoan, Paramecium, to very weak horizontal magnetic fields. Biol. Bull., 1962, 123, 164-181.

(1) The orientational response of the planarian, Dugesia, at a given time of solar day undergoes what appears to be a semi-monthly or monthly fluctuation, probably a consequence of the possession of a lunar-day rhythm in response to some compass-directional factor.

(2) The monthly rhythm of the planarian, Dugesia, is modifiable by a weak magnetic field.

(3) The monthly rhythm appears to undergo an annual modulation.

(4) Dugesia exhibits a response to weak magnetic fields in the range of 0.17 to 10 gauss.

(5) Dugesia differentiates between a horizontal field parallel to the long axis of the body and a field at right angles, and between N and S poles, and, furthermore, is able to resolve intermediate angular orientations of field with remarkable precision.

(6) The response of Dugesia alters its character in passing from a field close to the earth's strength to one as little as 10 gauss, suggesting the perceptive mechanism to be specifically adapted to such a weak field as the geomagnetic one.

(7) There is suggestive evidence that the protozoan Paramecium also responds to very weak magnetic fields.

(8) Some possible roles for organisms of such astounding responsiveness to very weak magnetic fields are discussed briefly.

38. Brown, F. A. Response of the planarian, Dugesia, to very weak horizontal electrostatic fields. Biol. Bull., 1962, 123, 282-294.

(1) The planarian Dugesia is able, even while in water, to perceive a change of 2 volts/cm. in electrostatic gradient in the surrounding air.

(2) There is reason to presume that in order to show this response the organism is responding to differences in ambient static gradient of the order of fractions of a micro-volt per cm.

(3) The strength and character of worm response to a right-angle potential change are related to the direction the worm is oriented in the earth's geographic field, and to time of day.

(4) A field-change in South-bound worms in the morning effects clockwise turning. A similar field-change for North-bound worms effects counterclockwise turning. In the afternoon the relationship of electrostatic response to geographic direction is essentially the mirror-image of that of the morning.

(5) Dugesia is able to distinguish the direction of a gradient across its body.

(6) A few of the possible significances of these findings are discussed briefly.

39. Brown, Frank A., Jr. An orientational response to weak Gamma radiation. Biol. Bull., 1963, 125, 206-225.

(1) The common planarian worm, Dugesia dorotocephala, displays a significant orientational response to increase in Cs^{137} gamma radiation when the increase is no greater than 6 times background.

(2) The worms are able to distinguish the direction of the weak gamma source, turning away from it, whether it is presented on the right or left side. The response sign is, therefore, the same as that of the response of these negatively phototactic worms to visible light.

(3) There is a clear compass-directional relationship of the responsiveness to the experimental gamma radiation. A conspicuous negative response is present when the worms are traveling northward or southward in the earth's field with the gamma change in an east-west axis. No statistically significant mean turning response to the gamma radiation is found when the worms are traveling eastward or westward in the earth's field with the gamma change in a north-south axis.

(4) The previously observed annual fluctuation in the character of the monthly orientational rhythm of north-directed worms has been confirmed in an additional year of study. During colder months, the rhythm is monthly; during warmer months it is semi-monthly.

(5) There is a semi-monthly fluctuation in the response of Dugesia to weak gamma radiation during mid-morning hours, the worms turning away from the source for four days prior to new and full moon, and toward it for two days following new and full moon. The stronger the field strength, up to 9 times background, the larger the amplitude of the rhythm.

(6) There is a direct relationship between intensities of gamma radiation between that of background and 9 times background, and the strength of the negative response of the worms.

(7) Evidence suggests that the negative response of Dugesia to gamma source may be modified by experimental alteration of the natural ambient electrostatic field.

(8) Some possible biological significances of this remarkable responsiveness to gamma radiation, and its particular properties, are discussed briefly.

40. Brown, F. A., jr. & Park, Y. L. H. Seasonal variations in sign and strength of Gamma-taxis in planarians. Nature, 1964, 202, 469-471.

The evidence presented here, together with that offered earlier, clearly indicates that an informational input reflecting changes in the external environment is available in terms of gamma radiation to at least two widely different kinds of organisms, mud-snails and planarians, and probably to living things in general. Along with such other stimuli such as light, sound, and odors, therefore, gamma radiation is a potential modifier of biological systems and activities. No attempt has been made as yet to discover possible normal ecological, physiological, or behavioral significances of this newly disclosed sensory inflow. It could, conceivably play subtle but important parts in still unexplained aspects of such phenomena as biological clocks, animal homing and navigation, and human behavior.

41. Best, J. B. Diurnal cycles and cannibalism in planaria. Science, 1960, 131, 1884-1885.

Dugesia tigrina attacks Cura foremanii under certain circumstances. The length of fast prior to the attack and diurnal rhythms are involved in evoking such behavior.

42. Best, J. B., & Rubenstein, I. Environmental familiarity and feeding in the planarian. Science, 1962, 135, 916-918.

Planarians, in common with higher animals, tend to delay feeding in environmental conditions to which they are not habituated.

43. Parker, G. H. & Burnett, F. L. The reactions of planaria with and without eyes to light. Amer. J. Physiol., 1900, 4, 373-385.

Planarians without eyes react to the directive influence of light in much the same way as those with eyes, in that they have a tendency to turn away from the course when directed toward the source of light and to keep in it when directed away from the source, though with less precision and often to less extent than planarians with eyes.

Planarians with eyes move more rapidly (1.12 mm. to 1.04 mm. per sec.) than those without eyes (0.89 mm. to 0.82 mm. per sec.); and those moving away from the light (1.12 mm. and 0.89 mm. per sec.) than those moving toward it (1.04 mm. and 0.82 mm. per sec.).

44. Walter, H. E. The reactions of planarians to light. J. Exp. Zool., 1907-8, 5, 35-162.

Light is not an essential factor in planarian activities, since the behavior necessary to the welfare of the individual and the race is mainly referable to other factors.

A planarian's response to light is of a passive character, which may have an adaptive significance only in so far as its phototaxis tends to conceal the worm from its enemies. The presence of pigment may also be regarded as an adaptive condition induced by the animal's relation to light.

The evolution of the photoreceptive apparatus of the planarian has not reached the degree of differentiation necessary to enable it to secure for itself such adaptations to the factor of light in its environment as would make aggressive activity possible to it in a manner characteristic of higher animals.

45. Taliaferro, W. H. Reactions to light in Planaria maculata. J. exp. Zool., 1920, 31, 59-116.

The eye of Planaria maculata is a typical turbellarian eye, consisting of two types of cells--the accessory cells forming the pigment-cut and the sensory cells or retinulae.

Each retinula consists of three regions--the nucleus-bearing region, the middle region, and the rhabdome, which show a striking resemblance to the three regions of the vertebrate retinula, viz., the myoid, the ellipsoid, and the rhabdome.

Planaria maculata is negative to light and orients accurately to a horizontal beam of light.

Specimens with both eyes removed do not orient in directive illumination as do normal specimens. They move, however, in general, away from the light.

Removal of both eyes does not appreciably affect the rate of locomotion in either directive or non-directive illumination.

Removal of the anterior end, on the contrary, greatly retards the rate of locomotion in both directive and non-directive illumination.

Specimens with one eye removed show no indication of circus movements or other abnormal motor activities.

Specimens with one eye removed orient accurately to light, when illuminated on the normal side, by turning directly away from the source of light.

Such specimens do not orient to light when illuminated on the 'blind' side unless the head is moved so that light enters the remaining eye.

The rhabdomes in the eye are arranged in two localized sensory regions; illumination of the rhabdomes of the posterior and ventral edge of the pigment-cut is followed by the animal's turning toward the side containing the eye, while illumination of the remaining rhabdomes is followed by the animal's turning in the opposite direction.

Light must strike a given rhabdome parallel with its longitudinal axis in order to cause stimulation of the rhabdome. Thus the position of the longitudinal axis of the rhabdomes results in a localization of photic stimulation.

(excerpts from the author's summary)

46. Thiemann, W. Die Phototaxis als Präferendumernstillung-Versuche an Planaria gonocephala Duges. Zool. Jahrb., 1957, 62, 177-208.

Planarians placed in a light gradient from 0.3 to 20 Lux selected the region 1.7 to 9 Lux and showed avoiding behavior at the boundaries of this region. Anterior ends react like whole animals or even more definitely, while decapitated worms show no orientation. Removal of the auricles caused a uniform distribution of the animals in the

lighter part of the gradient. In a stronger gradient, 4-150 Lux, intact worms sought the same part of the gradient as before to the extent this was possible; bilateral blinding was without influence on the behavior. Anterior ends showed marked tendency to seek the darker part of the gradient and decapitated worms exhibited no orientation. In a still stronger gradient, with strongest light at the two ends, decreasing to the middle (346-135-355 Lux), the worms gathered in the middle where the light intensity was least. In a weak gradient, 0.1 to 6.5 Lux, the worms still showed preference for the weakest part of the gradient but eventually came to rest at the ends of the apparatus, a thigmotactic response. In ultraviolet, the parts of the gradient farthest from the light source was sought; blinded animals showed less sensitivity to UV and decapitated worms did not orient. With a single light, no preference could be found. Rate of locomotion is greater when the anterior rather than the posterior end is illuminated and the same result obtained for blinded animals with or without the brain, but the speed is lessened. If the auricles are removed, the speed of locomotion is greater when the posterior end is illuminated. In a light gradient, speed is diminished in the preferred area.

47. Behrens, M. E. The electrical response of the planarian photoreceptor. Comp. Biochem. and Physiol., 1962, 5, 129-138.

Slow negative potential changes in response to illumination have been recorded extracellularly through micropipette electrodes which penetrated the eyespots of the planarian, Dugesia tigrina. As in other photoreceptors the response amplitude increased and the latent period decreased with increasing stimulus intensity. Dark adaptation was half complete in 3 or 4 minutes. A transient decrease in amplitude complicated the response of the dark adapted eye to a high-intensity stimulus. A small positive deflection or a notch was present at the beginning of some responses. Occasionally a shift in baseline and polarity reversal were observed in association with vertical electrode displacement. These events might be related to penetration of the membrane covering the pigment cup.

48. Brown, H. M., Jr. Photoreversal of the light-response decrement in planaria due to ultraviolet light. Am. Psych., 1964, 19, 484 (Abstract).

The purpose of this study was to determine the effects of 2,600 Å and photorestorative light, known to affect cellular nucleic acids, on the behavior of planaria. Nine groups of conditioned and naive worms were used. Three groups each received either ultraviolet light or ultraviolet plus photorestorative light and three groups were used as controls. Worms in 6 of the groups were bisected and allowed to regenerate 2 wk. Results disclosed that worms exposed to ultraviolet light alone took more extinction trials than controls or those exposed to ultraviolet and photorestorative light. However, tails consistently showed the greatest response decrement forcing the interpretation that short regeneration periods and lower light intensities (480 ft-c) used to test retention reveal intrinsic differences in head and tail regenerates and can confound treatment variables.

GRAFTING

1. Okada, Y. K., & Sugino, H. Transplantation experiments in planaria. Jap. J. Zool., 1937, 7, 373-439.

(1) Small square or rectangular pieces were cut from different levels of a planarian and transplanted into the same (autoplastic transplantation) or another individual (homoplastic t.) at different levels either in normal or reversed orientation. The object of this

series of experiments was to determine the degrees of specialization from the head to the tail end of the animal. On the other hand, parts were united, which were thus known to be provided with different specialization, in the hope that some new light might be thrown on the problem above discussed as to the various influences on the host. In such cases it is evident that the graft itself will be influenced by the host according to the level of transplantation. It is also necessary in these experiments to take into consideration the effect of quantitative difference between the sizes of the graft and the host.

(2) Hence, two pieces of comparatively large size cut out at the same or at different levels of the body were united in normal or reversed orientation, and in the light of these experiments the results of the preceding experiments of transplantation of small pieces were criticized. Again, subsequent cuts were performed in these experiments for the purpose of investigating that in what manner the difference in size and degree of specialization between the components of the compound body determine in particular the polarity of the regenerated part of the operated surface.

(3) Homopleural or heteropleural reindividualization was made with lateral halves of two worms which were longitudinally split. This implies combination of the results of the first series of experiments with those of the second, representing the union of two halves with equal or unequal differentiation along a wide range from one end to the other of the worms, with the aim of elucidating the mechanism of subsequent reorganization of tissue and the formation of new organs.

2. Sugino, Hisao. Miscellany on planaria transplantation. A supplemental note to the transplantation experiments in planaria Dugesia gonocephala. Annot. Zool. Japon., 1938, 17, no. 2.

(1) When a large median part of the planarian body is cut out, rotated 180 degrees, and replaced in the same place, dorsal elevations are produced at the anterior and posterior ends of the rotated part with corresponding ventral invaginations. Two pharynges develop, one in the anterior new tissue and the other in the postpharyngeal region of the host in normal orientation. The polarity of new tissue always directs from the originally anterior to the posterior of the two components united.

(2) When a median large part as above is transplanted into a corresponding level of another worm in normal orientation, a perfect worm results.

(3) When two worms from which in one the right margin and in the other the left margin have been removed, are united side by side by their cut-surfaces, a single worm with double internal structure is formed.

(4) When the lateral tissue strips after removal of the median large part of the body are brought to unite along the interior cut-surfaces, new tissue does not appear along the line of union. The worm does not recover the width but continues to increase in length.

3. Sugino, Hisao. Homopolar union in Planaria gonocephala. Jap. J. Zool., 1941, 9, no. 2.

(1) Pieces taken from different levels of Planaria gonocephala are united in homopolar orientation.

(2) When a comparatively large rectangular piece of the prepharyngeal region is transplanted into the pharyngeal region of other worm in normal orientation, slight elevation occurs at the anterior border of union, and in the host tissue is produced a pharynx in reversed direction; the polarity of the region near the line of union is reversed.

(3) When two prepharyngeal pieces are united in homopolar orientation, new tissue slightly appears along the line of union, the polarity of which is reversed.

(4) When the posterior cut surface of the postpharyngeal region and the anterior cut surface of the pharyngeal region are united, a head and a tail regenerate respectively on the anterior and posterior end, and new tissue is produced along the line of union, the polarity of which and even of the neighboring tissue is reversed.

(5) When three pieces from different levels of the same animal are united so as to arrange originally the most posterior piece in the most anterior situation and the most anterior piece in the most posterior position, a head regenerates on the anterior and a tail on the posterior end, and at the line of union of each piece new tissue develops with reversed polarity. In each united piece a pharynx develops in normal direction; reversed pharynx also often appears according to the level from which the piece is taken.

4. Sugino, Hisao. Recombination experiments of small pieces in Dugesia gonocephala (Duges) Memoirs of the Osaka Univ. of the Lib. Arts and Educ. -B Nat. Sci., 1953, no. 2.

(1) The body of Dugesia gonocephala (Duges) was sectioned into short pieces of about 1 mm in length, and these were united in a converted or in their original position.

(2) In the first series of experiments, pieces of posterior level were arranged anterior to those of anterior level in the regular order, all pieces being in the normal orientation. Various combinations were made by uniting from two to sixteen pieces.

(3) In all cases, a slight new tissue was produced at each line of union of the pieces, the polarity of new tissue being reversed.

(4) In the unions of small number of pieces, sometimes entirely normal worms were formed.

(5) The polarity of the head pieces, especially between two eye spots, was never altered, unless they were macerated and the tissue was reorganized as a whole.

(6) The pieces taken from the posterior extremity of the worm developed head or tail from their anterior cut surfaces when they were united by the posterior cut surfaces with the pieces taken from the more anterior levels.

(7) In the second series, a head piece was inserted between pieces of posterior levels. The head thus inserted could not inhibit the regeneration of new head on the fresh anterior cut surface lying several millimeters apart.

(8) In the third series, pieces from various levels were combined in the irregular order, each piece being arranged in the normal orientation.

Prepharyngeal pieces placed posterior to those taken from the distant posterior regions also could not inhibit the regeneration of new head on the anterior cut surface of the anterior component.

(9) In the fourth series, alternate union of prepharyngeal pieces with those taken from pharyngeal and postpharyngeal regions was tried. In one case, in which five prepharyngeal pieces (including a head piece) were united, each in the reversed orientation, alternately with pharyngeal and postpharyngeal pieces. Two pharynges in the opposite directions were formed in each of the postpharyngeal components, and one in the normal orientation in the pharyngeal component, and all the pharynges presented reaction when chicken liver was given as food. Induction of the development of pharynx or pharynges by the prepharyngeal pieces and the direction of pharynges may be clearly explained by this example.

(10) Pieces cut in a varying length and taken from different regions were united in the normal orientation and in the original order. Most of them gave rise to normal worms. The results show that the effect of the cutting and the delay of the union are by no means the cause of proliferation of a new tissue, still less the new center of reorganization.

(11) The development of multiple pharynges was very often observed in the worms derived from the united pieces, the direction and position of which being determined by the regenerative capacity of one piece and organizing power of the other united.

(12) In conclusion, any piece taken from the planarian body acts as an organizer upon the pieces taken from the more posterior levels, at the same time it is reorganized by the pieces taken from the more anterior levels.

5. Miller, J. A. Studies on heteroplastic transplantation in triclads. I. Cephalic grafts between Euplanaria dorotocephala and E. tigrina. Physiol. Zool., 1938, 11, 214-247.

A technique of operation involving rectangular transplants and ice as anesthetic gave a high percentage of takes between E. dorotocephala and E. tigrina. Four hundred and twelve cases of successful implantation of cephalic grafts were studied. Of these, 105 were in the head, 113 in the pharyngeal level, and 174 in the tail. Twenty were placed in the anterior prepharyngeal region for comparative purposes.

In the discussion the following topics are treated: (a) triangular versus rectangular grafts, (b) polarity, (c) replacement of graft by host tissue, (d) graft migration, (e) induction and range of dominance, and (f) role of the nervous system in planarian induction. (excerpts from author's summary)

6. Lindh, N. O. Heteroplastic transplantation of transversal body sections in flatworms. Arkiv Zool., 1959, 12, 183-195.

In some cases heteroplastic transplantation can be performed also in flatworms. During the first day the connection between the joined parts was rather loose, but later the bonds were gradually strengthened. The affinity between the cells of the two strains is tissue-specific and to some degree level-specific, i. e., transversal parts from different longitudinal levels are the more difficult to graft the further apart their original position. At the common internal wound the fixation of the tissues was primarily made by differentiated tissue cells. When parts from different longitudinal levels were joined, the neoblasts gradually intermingled after a determination and differentiation. In spite of the movable cells migrating all over the resulting new unit, a mosaic was never formed.

The resulting mixed interstitial fluid in combination with membrane reactions in the area of conjunction (the internal wound) provoked the formation of a peculiar tissue barrier between the parts. This barrier, which consisted of extracellular collagenous structures and had a great aggregation of pigmented cells, constituted when fully developed a hindrance for the movable cells; consequently the old chimera had pure lines of cells in their two parts. Notwithstanding this it is incorrect to regard the mixed animals as parabionts because of the common nerve system and properly arranged internal organs.

From a histologic point of view the combination of tissues from different strains resulted only in a slight increase of basophilia and acid polysaccharides, whereas the content of neutral polysaccharides and basic proteins showed a marked decrease. All these facts signified a mutual influence of the grafts and may be regarded as general rejuvenation of the chimeric unit. When regeneration was enforced on a mixed animal, the mitotic activity reached the level of that of a young animal, which was a further proof of rejuvenating processes caused by the heteroplastic transplantation.

7. Teshirogi, Wataru. Transplantation experiments of two short pieces of a freshwater planarian, Bdellocephala brunnea. Jap. J. Zool., 1963, 14, 21-48.

(1) Two short pieces taken from the prepharyngeal region or the prepharyngeal and the postpharyngeal regions of Bdellocephala brunnea were united in the various combinations.

(2) When these two pieces were united in any orientation or in any arrangement, the regeneration at the anterior and the posterior free cut-surfaces of the combined worm was determined according to the original regenerating potency in each level.

(3) Regeneration between the two components was inhibited if the cut-surfaces were placed together in such a way that the pieces with opposite polarity and the same level of the potential gradient met. In this case, however, if the union was made a certain time after the cutting, the development of a vertical pharynx of one or two head-outgrowths on the line of union was observed.

(4) On the contrary, if two pieces were united from different levels of potential gradient, new tissue was invariably formed between them. In several combined worms a pharynx was induced in the new tissue or the development of one or two head-outgrowths near the line of union was observed. If the pieces were united after a certain interval after the cutting, a high rate of pharynx and head-outgrowth formation was observed in the combined worms.

(5) On the basis of these findings the nature of the new tissue in the combined worm has been discussed in detail.

MORPHOLOGY

1. Betchaku, T. A copper sulfate-silver nitrate method for nerve fibers of planarians. Stain technology, 1960, 35, 215-218.

For staining in toto, planarians are fixed in a mixture of 10 ml of commercial formalin, 45 ml of 95% ethanol and 2 ml of glacial acetic acid. After treatment with 70% ethanol 3-10 days, they are washed in distilled water and immersed in 10% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ for 3 hr at 50°C, transferred without washing to 1% AgNO_3 for 1.0-1.5 hrs at 50°C; and then developed in: 10 ml of 1% pyrogallol, 100 ml of 56% ethanol and 1 ml of 0.2% nitric acid. Gold toning, 5% $\text{Na}_2\text{S}_2\text{O}_3$ and dehydration follow as usual. For staining sections, material is fixed in the same fixative, embedded in paraffin and sectioned at 10 μ . After bringing sections to water, they are immersed in 20% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ for 48 hr at 37°C; then rinsed briefly in distilled water and placed in 7% AgNO_3 for 24 hr at 37°C. They are washed briefly in distilled water and reduced in: hydroquinone, 1 gm; Na_2SO_3 , 5 gm and distilled water 100 ml. Gold toning, followed by 5% $\text{Na}_2\text{S}_2\text{O}_2$ and dehydration completes the process. Any counterstaining may follow.

2. Gazsó, L.R., Török, L.J., & Rappay, G.Y. Contributions to the histochemistry of the nervous system of planarians. Acta Biologica, 1961, 11, 411-428.

In the methodical part of their investigations authors have searched for such staining methods as are suitable to stain electively enough nervous elements of the planarians, being simple and quick at the same time. They have found that, among the known staining methods, the HANSEN molybdate-hemotoxylin as well as MALLORY and GIEMSA stainings are most suitable to stain intensively the nervous fibers and nerve endings, however, the electivity of these methods is not sufficient. One can obtain a

good and almost elective nerve-staining on slides after steeping with a solution of 4% ammonium-molybdate and staining with a thionin solution (or that of some other basic stains) of a very low concentration. For all these staining methods, the ZENKER fixation was found as most suitable. The enzyme histochemical investigations have been made with the aim of searching for suitable histochemical methods for the morphological study of nerve elements, on the one hand, as well as of finding such ones for the registration of their functional state, on the other. In two fresh-water planarian species (Dugesia lugubris and Dendrocoelum lacteum), the alkaline phosphatase and specific cholinesterase activity of the nervous system was studied by using the methods of GOMORI and MENTEN--JUNGE--GREEN, respectively that of KOELLE--FRIED--ENWALD. As for Dugesia lugubris, positive alkaline phosphatase reaction was found in the whole central nervous system, with localization in the nuclear membranes, nucleoli and nerve fibrils of the nerve cells. Positive reaction was found also in the motor nerve fibers establishing the neuromuscular connexions. Here, the reaction was much more intensive as in the central nerve fibers. Images got by the GOMORI method are suitable also for studying the morphology of the nerve elements, as well as for distinguishing from each other motory and sensory fibers, respectively their endings. Authors suppose the alkaline phosphatases to play an important role in neuromuscular transfer of nerve impulses. As for specific cholinesterase, positive reaction was only found at the somatic neuro-muscular junctions, with localization in the motory nerve endings. A striking lack for positive cholinesterase reaction in the pharynx may be explained, in the authors' opinion, by morphological and physiological differences in the innervation of the somatic and visceral musculature. In the Dendrocoelum lacteum, authors could find, surprisingly enough, no positive reaction either for alkaline phosphatase, or for cholinesterase. Taking into consideration this fact, as well as the results of investigations performed in other directions, the authors suppose the much weaker regenerative capacity of that species, in comparison with Dugesia lugubris, to be connected with biochemical and physiological differences existing between nerve cells of the central nervous system of the two species.

3. MacRae, E. K. Observations on the fine structure of photoreceptor cells in the planarian Dugesia tigrina. J. Ultrastructure Research, 1964, 10, 334-349.

The bipolar photoreceptor cell of the planarian Dugesia tigrina was studied by electron microscopy. The photosensitive structure within the pigment-enclosed eyecup is attached to the free end of a cell process. This process, the dendritic fiber, contains large vesicles, mitochondria, and numerous neurotubules. It leads into an expanded cell body located lateral to and outside of the opening of the eyecup. The cell body contains a nucleus, nucleolus, mitochondria, several Golgi complexes, multivesicular bodies vacuoles, free ribosomes, and ribosomes associated with an extensive endoplasmic reticulum longitudinally oriented in the cell. The axon, containing vesicles and few neurotubules, extends to the cerebral ganglion and ends in a terminal axonal enlargement which may contain mitochondria, numerous synaptic vesicles, and glycogen granules.

4. Milne, Lorus J. & Margery J. Invertebrate photoreceptors. Rad. Biol., 1956, 3, (Hollaender, A., ed.), Chapt. 14, 629-631.

The pages cited provide a short review of important research dealing with the photoreceptor cells in planarian eye spots. They refer to studies of the anatomy of these cells, of their responsiveness to chromatic and achromatic and to ultra violet light, to the role of the pigment cup in planarian vision. The balance of the chapter gives similar treatment to the photoreceptors of other invertebrates.

5. Pedersen, K. J. Cytological studies on the planarian neoblast. Z. Zellforsch., 1957, 50, 799-817.

The paper is a study of the cytology of the regeneration cells (neoblasts) in planaria vitta.

The morphology of the living cells has first been examined to provide a reference for an investigation of the fixed neoblasts as studied by ordinary cytological, cytochemical and electron microscopical techniques.

A rather selective staining method has been devised based on the basophilic properties of the scanty cytoplasm. The morphology of the fixed neoblasts and their distribution in the intact animal have been described, using this method.

The marked cytoplasmic basophilia was found to be exclusively due to ribonucleic acid, and not to desoxyribonucleic acid or acid mucopolysaccharides.

The cytoplasm contains moderate to considerable amounts of basic proteins. Tyrosine, cysteine/cystin, arginine, lysine and perhaps histidine were present, while tryptophan could not be demonstrated.

No enzymes could be demonstrated apart perhaps from cytochrome oxidase.

The mitochondria are small and inconspicuous and more or less evenly distributed throughout the cytoplasm. A Golgi apparatus could not be demonstrated.

The electron microscopical picture is very characteristic, because of the high electron density of the cytoplasm. This density is the result of the presence of a great number of ribonucleoprotein granules. Most of the granules are free and only a minor part bound to the membranes of the endoplasmic reticulum. The interesting feature of the cell membrane are discussed in relation to the structure of the parenchyma.

The cytochemical properties of the neoblast (RNA and sulfhydryl-group containing protein) and the fine structure as revealed in the electron microscope characterize the neoblast as morphogenetically active cell.

6. Pedersen, K. J. Studies on the nature of planarian connective tissue. Z. Zellforsch., 1961, 53, 569-608.

Cytological, histochemical, electronmicroscopical and in vivo dissociation methods have been employed in studies on the connective tissue in two planarian species. Two cell types belong to the connective tissue: the "free" neoblast and the "fixed" parenchyma cell.

The fixed parenchyma cells penetrate and bind together all structures between the epidermis and gastrodermis. The cells are very large and provided with many attenuated processes. The cytoplasm exhibits a strong PAS-reaction, presumably due to a high content of neutral polysaccharides. The cytoplasmic RNA is only present in small quantity. There are indications of very low activities of oxidative enzymes and hydrolases. Correlated light microscopical and electron-microscopical examination revealed that most of the PAS-reactive material is intracellular.

In electronmicrographs the cytoplasm of fixed parenchymal cells is generally pale and contains few, irregularly distributed mitochondria and scanty Golgi material. In Planaria vitta a large number of lipid droplets are present. This is not the case in Dugesia tigrina. Many vesicles of several types are observed. Also the occurrence of lysosome-like structures is a characteristic finding.

The fixed parenchyma cells do not form a syneytium, and desmosomosomal contacts with other cells have not been observed. The extracellular space is extremely small, limited to the narrow space between two opposing cell membranes. In few places it is slightly widened. Little or no ground substance is present.

Connective tissue filaments are found in the highly developed subepidermal basement membrane. The finding of filaments in many portions of the narrow intercellular

space is of considerable interest.

The great flexibility and probably great functional significance of the fixed parenchyma cells is discussed in relation to modern concepts of cellular transport mechanisms.

It is suggested that the connective tissue in planarians perhaps is one of the most primitive expressions of connective tissue in the animal kingdom.

PHYSIOLOGY AND REGENERATION

1. Brøndsted, H. V. Planarian regeneration. Biol. Rev., 1955, 30, 65-126.

(1) T. H. Morgan was the first to merge the problems of planarian regeneration into the science of morphogenesis.

(2) C. M. Child was the first to introduce quantitative methods into this field. He and his co-workers demonstrated the phenomenon of head-frequency, i. e., an ability to regenerate head, graded in a species-specific way along the main axis of the body.

(3) I have extended this notion as follows: there exists a time-graded regeneration field tapering antero-caudad and medio-laterad. Every wound or cut exposes a surface in which there will always be a "high-point" where regeneration starts earliest; from here forces travel to other parts of the blastema inhibiting these from regenerating the same structure as the "high-point". The material basis of the time-graded regeneration field is unknown. The regeneration rate of every point in the field is firmly fixed as a species-specific characteristic.

(4) Wolff & Dubois have conclusively shown, by an ingenious X-ray technique, that the cellular material forming the blastema is made up of totipotent neoblasts migrating from the interior of the body.

(5) The problem of dedifferentiation of cells is not solved; therefore the processes involved in morphallaxis, the remoulding of old tissues, are unknown. Reindividualization of heteromorphs involves problems of the same kind; it is thus not understood.

(6) The relationship of asexual reproduction to regeneration is a close one, but nothing definite is known; for this reason morphological, histochemical and cytochemical investigations are greatly needed.

(7) Polarity may be reversed, especially in short pieces, e. g., "Janus-heads".

(8) The head-frequency phenomenon induced Child to formulate his much-debated hypothesis of gradient metabolism, in which he states that the level of metabolism is decisive for morphogenesis: 'The axial gradients in rate of reaction constitute the basis of polarity and symmetry in the organisms.' Later work on planarians did not, however, substantiate his claims.

(9) The totipotent, embryonic neoblasts are the cells most sensitive to irradiation.

(10) Rate of regeneration is not decisively influenced by the size of piece; this rate depends on forces in the time-graded regeneration field.

(11) Prolonged starvation retards the rate of regeneration.

(12) Regeneration is inhibited if a wound is closed in such a way that pieces with opposite polarity meet one another.

(13) Wolff & Lender have shown that eye formation in competent tissue is dependent on the presence of the head ganglion. Apart from this observation, the role played by the nervous system in planarian regeneration is still unsolved.

(14) An adult head does not act as an 'organizer'.

(15) Neoblasts contain ribonucleic acid. Rate of regeneration is accelerated by ribonucleic acid in starved planarians. SH-groups are probably of special importance in regeneration. Glycine, arginine, cystine and histidine are claimed to accelerate regeneration. Riboflavin accelerates regeneration.

(16) Rate of respiration does not follow the head-frequency curve. Respiration of the blastema is largely unknown.

(17) The following working hypothesis is proposed. After removal of a body-part, the rest 'determines' the fate of the blastema in this way: the totipotent neoblasts start at a given level in the time-graded regeneration field; they regenerate head or tail respectively until morphogenesis by differentiation of the neoblasts has filled up the lacking biochemical niches; then the neoblasts are inhibited from going further by the serological factors differentiated in the already existing adult tissues. The morphogenetic equilibrium is established by finer adjustments of a serological kind from cell to cell. No freely diffusing 'Organisatorstoffe' have been found. Inhibitory influences from the 'high-point' in the blastema traverse the blastema laterally, in 80-90 hr., in an anterior head-blastema of Dendrocoelum.

2. Bardeen, C. R. On the physiology of the Planaria maculata, with especial reference to the phenomena of regeneration. Am. J. Physiol., 1901, 5, 1-55.

One of the classic definitive observational studies on the course of regenerative processes in the planarian, P. maculata. The article includes descriptions of regeneration after a number of cuts at different loci in the animals, particularly as related to the intestines, and stressing the importance of the axial gut. It is instructive to read his theorizing, made in the light of what was known in the area at that time. It illustrates rather cogently the misinterpretations possible when an imaginative scientist has just a little information. But it serves also to remind us that much of what we laboriously search out by experimental data collecting today may be sitting, ready made, for us if we but take the time to read about the reputable experimentation of those who passed this way before us. It's also surprising to discover that on many important theoretical points, we've progressed not an inch in eliminating alternatives - even with all our technological miracles. Some of the same old arguments are still with us - full blast.

3. Buchanan, J. W. An analysis of physiological states responsible for antero-posterior disintegration in Planaria dorotocephala. Protoplasma, 1935, 22, 497-512.

Planaria were treated with equi-molal solutions of ammonium, potassium, sodium, magnesium, and calcium chlorides, made up in distilled water and the rates of cytolysis compared with cytolysis in distilled water. Potassium and ammonium accelerate cytolysis; some protection is afforded by sodium; still more by magnesium, and complete protection by calcium in the concentrations employed.

In distilled water solutions of calcium chloride no cytolysis occurs in concentrations from M/500 to M/40,000; cytolysis is distinctly delayed in M/100,000. The protective action of M/1,000,000 is detectable.

Potassium oxalate accelerates disintegration in hypotonic solutions.

One per cent ethyl alcohol in distilled water causes cytolysis more rapidly than does distilled water alone, but in M/500 molal calcium chloride the alcohol solution is much less effective.

Ringer's solution minus calcium affords no protection against death due to absence of calcium and death due to potassium oxalate but completely protects against cytolysis. Death in Ringer's solution minus calcium and in Ringer's solution with potassium oxalate occurs first in the anterior region and describes an antero-posterior gradient.

Cytolysis in distilled water, in potassium oxalate solutions, in alcohol solutions, and in hypotonic calcium solutions of extreme dilution is initiated in the anterior end and describes an antero-posterior gradient within a zoid.

Earlier work of the writer on the disintegrative action of lipid solvents, head, KNC, hyper- and hypotonic solutions is discussed. It is concluded that in Planaria

dorotocephala the antero-posterior gradient in cytolytic disintegration represents an antero-posterior differential in sensitivity to disturbance of the calcium-lipoid-water relation in the organism.

4. Buchanan, J. William. The effect of planarian extracts and exudates upon head regeneration in Euplanaria dorotocephala. Physiol. Zool., 1938, 11, 144-154.

Water extracts of freshly cut planarian heads induce increases in the number of heads regenerated by isolated cross-pieces of E. dorotocephala when such extracts are applied for periods less than 24 hours immediately after the sections have been cut.

Head extracts when introduced into the intact animal in a posterior region, or when applied to animals injured in a posterior region, do not induce head formation at the site of injury.

If living heads or posterior ends are included with regenerating pieces in small volumes of water, 0.2 cc., for less than twenty-four hours after cutting, the regenerates show the effects of crowding. In some cases head frequency is increased and in others it is decreased by such treatments.

If the crowding is less intense but continues throughout the regeneration period there is a tendency for head frequency to increase.

Evidence of an effect on head frequency of living planarian heads separated from the regenerates by glass or quartz walls was entirely negative.

These results when considered together with the fact that many other agents and conditions alter head frequency, e. g., low temperature, organic and inorganic acids, bases, anesthetics, and alkaloids, support the conclusion that whether or not a head regenerates in a piece of E. dorotocephala depends upon quantitative physiological conditions within the piece; specific action of particular agents cannot be called upon to explain the experimental results.

5. Wiercinski, Floyd J. The effects of supersonic vibrations on reconstitution and head frequency in Euplanaria dorotocephala. Physiol. Zool. 1939, 12, 62-69.

(1) With a certain range of intensity and duration of exposure, supersonics bring about cytolysis in whole individuals of E. dorotocephala. This cytolysis progresses from the anterior end posteriorly in the anterior zooid, just as is the case when the worms are exposed to certain other physical and chemical agents.

(2) On return to water after partial cytolysis, reconstitution occurs.

(3) Worms exposed in groups of few individuals are more susceptible than in groups of many individuals.

(4) In general the effect increases with intensity and duration of exposure, but individual differences occur as with other agents.

(5) For determination of the effect on head frequency, experimental and control lots of pieces A, B, C, and D (approximately successive fourths of the anterior zooid) were used, since these pieces give progressive decrease and a wide range of head frequency from A to D. Effective intensities and exposure times were determined by preliminary experiment. Lots were exposed immediately after section and following various periods of delay.

(6) With exposure immediately after section little change in head frequency occurred.

6. Dubois, F. Contribution à l'étude de la migration des cellules de régénération chez les planaires dulcicoles. Bull. Biologique, 1949, 83, 1-71.

(1) The planarians used are Euplanaria lugubris, Euplanaria maculata, and Polycelis nigra. The experimental methods used are localized irradiation (the most often

irradiation of the anterior two fifths of the body with subsequent section behind the eyes), and irradiation combined with transplantation.

(2) The E. lugubris are normally endowed with great power of regeneration. The rate of regeneration diminishes at lower temperatures and when the section is performed in the posterior region of the body of the animal.

(3) Planarians submitted to a dose of X-rays of greater than 3500 roentgens are incapable of regenerating and die three to five weeks after irradiation. Planarians locally irradiated but left whole die.

(4) The E. lugubris irradiated ahead of the pharynx and amputated behind the eyes regenerate after a period which corresponds to the duration of the migration of the cells coming from the healthy region toward the irradiated region. The cells of regeneration of neoblasts have, then, migrated through the whole irradiated region.

(5) The duration of regeneration is a function of the distance to be traversed by the neoblasts. Migrations exist from back to front as well as from front to back and from side to side in the planarian.

The rate of migration varies with temperature in the same manner as rate of normal regeneration. Migration exists also in E. maculata and in Polycelis nigra.

(6) The method of grafts combined with irradiation also proves the migration of the cells of regeneration. A rectangular transplant embedded in an irradiated host brings to him healthy neoblasts which migrate toward the head if the host is decapitated, and regenerates a head which takes the background color of the graft. The inverse experiment ends in the repair of the necroses of the graft. The organizing power of the planarian is not destroyed by X-rays.

(7) Migration does not unleash spontaneously. It must be brought forth by section. It is oriented toward this section, and cannot be directed toward another part.

(8) A simple incision not causing the formation of a blastema suffices to attract the neoblasts toward the affected region. There exists a system of interrelationships in the bodies of planarians. From the injured part goes a stimulus which is unaffected by X-rays and which sets in motion the migration of the neoblasts.

(9) The call to migration extends under normal conditions through the whole of the animal's body. The flow of neoblasts halts at the center of where the call to migration was set up by the lesion.

(10) The effective duration of the stimulus to migration is 4 to 5 days. A stimulus can affect neoblasts at rest or in the act of migrating, but the effect is lost as soon as the cells begin to differentiate.

7. Lender, Th. Le role inducteur du cerveau dans la régénération des yeux d'une planaire d'eau douce. Ann. Biol., 1952, 28, fasc. 9-10, 191-198.

On the whole the experimental results lead to the following conclusion: the brain is the inductor of the regeneration of the eyes of Polycelis nigra.

(a) The inductive action makes itself felt only in the receptive territory. This is normally limited to the ocular rim of the planarian.

(b) The inductive action is a characteristic of the brain. The regeneration of the eyes is impossible in the absence of the brain. If a third of the cerebral mass subsists, the regeneration of the eyes is possible. The inductive action of the brain exerts pressure on the nervous communications independently. The brain activates to a certain extent and not by contact with the neoblasts which differentiate into visual organs.

(c) The brain then seems to exert its inductive action through the intermediary of humoral substances or organisms.

The organisms are without doubt secreted by the nerve cells. They diffuse across the parenchyma. On the receptive border they regenerate into visual organs.

The organisms are not destroyed by X-rays. They are produced by nerve cells injured in the same way. Many of them have no taxonomic specificity.

8. Sengel, Philippe. Sur l'Induction d'une Zone Paryngienne chez la Planaire d'eau douce Dugesia lugubris O. Schm. Archives D'Anatomie Microscopique et de Morph. Exp., 1953, 42, no. 1.

(1) Experiments of transplantation have been carried out on the fresh-water planaria D. lugubris in order to be able to state precisely the inductive capacity of a cephalic transplant grafted into the postpharyngeal region of the planarian.

(2) A head graft is implanted into the concordant area or inversely into the post-pharyngeal of a planarian. At the end of 3 to 4 weeks, it is established that the graft has induced a supplementary pharynx in the tissues of the host. The polarity of the supplementary pharynx is always the same as that of the graft (i. e. orientation of supplementary pharynx to body is the same as that of normal pharynx.) Then the graft is cut out. It does not reject (the suppl. pharynx). The suppl. pharynx is extracted next. After 8 to 15 days, it is regenerated in its proper polarity that is, concordant or inverse in relation to the host.

(3) This is not a pharynx which the graft has induced in the host, but a whole pharyngeal region. This (region) retains in effect, the faculty to regenerate its pharynx in the absences of cephalic formations which have presided in its formation.

(4) These results are in agreement with the notion of the "zone of induction"; induction of a dominated zone, the pharyngeal region, by a dominant zone, the cephalic region. It is necessary to distinguish the induction of the zone of induction from (the induction of) distinct organs, as that which Th. Lender has observed in the regeneration of the eyes of P. nigra.

9. Lender, Th. Mise en evidence et proprietes de l'organisine de la régénération des yeux chez la planaire Polycelis nigra. Revue Suisse de Zool., 1955, 62, 268-275.

The existence of a regenerative organism of the eyes of the planarian P. nigra thus seems to be definitely established. It is chemical substance which diffuses in the water and across a gel, which is insensitive to the dessication and action of alcohol at 70 degrees. It does not have zoological specificity and remains active after color treatment. It is normally present in the anterior region of the body, the cerebral area, where it originates. In that area it must diffuse liberally into the tissues to act on the neoblasts which are found in the zone of regeneration of the cut-out ocular border. It is between the 3. and 5. day that the inductive organism reaches its maximum. The concentration of the organism does not change during the experimental regeneration, one can thus think that at that time the neoblasts are apt to react to the inductor and to differentiate into a visual organ. In the tail region, the organism can be tested by killing the tissues by heating the ground up animals to 60 degrees.

The experiments, done up to the present, again do not permit expressing an opinion on the chemical nature of the organism. But the achievement of obtaining the inductive substance in solution or in a suspension in water will permit entering upon the chief problem.

10. Lender, Th. Recherches experimentales sur la nature et les propriétés de l'induction de la régénération des yeux de la planaire Polycelis nigra. J. Embryol. exp. Morph., 1956, 4, 196-216.

(1) After excision of eyes and brain of the planarian Polycelis nigra (Ehrbg.),

regeneration of eyes fails unless the brain is allowed to regenerate too. But regeneration of eyes in the absence of the brain occurs if the animals are kept in the presence of a mince of planarian heads, or of such a mince embedded in agar, or of the supernatant obtained after grinding heads with sand. The induction of eye regeneration is therefore performed by a chemical substance, an organisine.

(2) Regeneration of eyes in the absence of the brain is possible if the organisine is only allowed to act on alternate days, or only for two consecutive days in all. In the latter case, best results are obtained if the substance acts during the 3rd and 4th days after operation.

(3) Regeneration of eyes is favoured by increased concentration or organisine in the medium.

(4) Treating the inducing material with 70 per cent alcohol does not inactivate the organisine, nor does warming to 60° for 2 minutes. Higher temperatures reduce its activity, and boiling for 30 minutes almost destroys it.

(5) The organisine is not species-specific. Its presence can be demonstrated in the brain region of Dugesia lugubris, D. gonocephala, and Dendrocoelum lacteum.

(6) A mince of the pharyngeal or caudal region does not produce regeneration of eyes. But a mince of the caudal region can be activated by treatment at 60° for 2 minutes. With stronger heating the inducing power diminishes, as with the mince of heads.

(7) A mince of caudal regions is also activated if it is treated with alcohol of 70 or 95 per cent. The supernatant obtained from grinding caudal regions with sand also contains the organisine.

(8) Such supernatants from the caudal regions are more active the greater the number of animals from which they are prepared; but they contain less organisine than the supernatant from an equivalent number of head regions.

(9) The regeneration of eyes of Polycelis nigra is therefore induced by a chemical substance, an organisine, present throughout the body of the planarian. Its concentration is stronger in the anterior than in the posterior region; in the latter it is furthermore immobilized within the cells. These results are comparable with those obtained in the study of amphibian embryonic induction.

11. Lender, Th. Analyse des phénomènes d'induction et d'inhibition dans la régénération des planaires. Ann. Biol., 1956, 32, fasc. 11-12, 457-471.

The analysis of the regeneration of the pharynx, the eyes and the brain of planarians puts in evidence three types of correlations:

(1) The regeneration of the pharynx is determined by a double induction. The cephalic region and the prepharyngeal region induce the differentiation of a pharyngeal zone. Then the regeneration of a pharynx is produced in the pharyngeal region without intervention of the anterior region of a planarian body. The dominant cephalic region induces a dominated zone: the pharyngeal region, capable of differentiating a pharynx. This is an example of zone induction.

(2) The regeneration of the eyes is induced by the brain. This induction is caused by a chemical process. The chemical substances secreted by the brain, "the organizers," normally cause the differentiation of the eyes in the ocular region. The second example shows that the differentiation of a definite organ depends on the activity of a chemical inducer.

(3) The brain secretes inhibiting substances which arrest the formation of other cerebral ganglia, that is, the regeneration of another head. There is then, a third type of humeral correlation in the planarians: the inhibition, by chemical processes, of regeneration of a definite organ.

(4) Thus the regeneration in planarians causes interference in a chain of humeral correlations.

12. Lender, Th. L'inhibition de la régénération du cerveau des planaires Polycelis nigra (Ehrb.) et Dugesia lugubris (O. Schm.) en présence de broyats de têtes ou de queues. Bull. Soc. Zool. de France, 1956, 81, 192.

(1) The planarians Polycelis nigra and Dugesia lugubris, after excision of the brain and eyes are kept in the presence of ground-up homoplastics of heads or of tails. One uses ground up animals or the remains of centrifuged ground up animals.

(2) In the presence of ground up heads, the eyes regenerate, but the differentiation of the brain is more or less strongly inhibited.

(3) In the presence of ground-up tails, the brain differentiates nearly as well as in the controls.

(4) The inhibition was due to a chemical substance, left in the body of the planarian, following a gradient decreasing from the head towards the tail. The substance diffuses in the water of the products of centrifugation. Its activity is specific.

13. Stéphen-Dubois, Françoise & Kilmayer, Simone. La migration et la différenciation des cellules de régénération chez la Planaire Dendrocoelum lacteum. Comptes rendus des séances de la Société de Biologie, 1959, 153, 1856.

The planarian D. lacteum is not normally endowed with the potential of cephalic regeneration when the transverse section of the amputation occurs behind the pharyngeal root. The pharyngeal and postpharyngeal regions meanwhile contain cells of regeneration capable not only of regenerating a tail region, but also of reconstituting a cephalic region after having gone over a distance which runs past 2/5 of the body of the worms sometimes. The cells have a total but latent histogenetic potentiality which reveals itself only if they are transplanted into a prepharyngeal region. Research is being done to try and discover the inhibiting factors which block the regeneration potential of the cells.

14. Sengel, Catherine. La région caudale d'une planaire est-elle capable d'induire la régénération d'un pharynx? J. Embryol. Exper., Morph., 1959, 7, Part 1, 73-85.

(1) Experiments involving amputation and irradiation have been made on the fresh-water planarians Dugesia lugubris and Polycelis nigra to ascertain the precise roles of head and tail in the induction of a pharyngeal zone and of a pharynx.

(2) During the normal regeneration of a prepharyngeal cephalic fragment, a pharynx regenerates in the original tissues of the fragment at the same time as the caudal blastema appears. During the normal regeneration of a postpharyngeal caudal fragment, the pharynx appears in the new tissues of the blastema. The eyes of the regenerated head have by then already appeared.

(3) The caudal blastemata of cephalic fragments were cut away every day or every second day. A pharynx appears after a few days in the fragment. The cephalic blastemata of caudal fragments were cut away every second day. No pharynx regenerated under these conditions.

(4) A zone close to the cut was irradiated with X-rays, which prevents the fragments regenerating for a long time. A pharynx appears in the cephalic fragment long before the regeneration blastema. In the caudal fragments, the pharynx cannot regenerate before the blastema has formed. It appears only after the blastema has differentiated into a head.

(5) The same results have been obtained in another species of planarian, Polycelis nigra.

(6) The regeneration of the pharynx is therefore possible in a cephalic fragment, devoid of any blastema. The regeneration of a pharynx is impossible in a tail without cephalic, prepharyngeal or pharyngeal regions.

15. Kolmayer, S. et Stéphan-Dubois, F. Néoblastes et limitation du pouvoir de régénération céphalique chez la planaire Dendrocoelum lacteum. J. Embryol. exper. Morph. 1960, 8, part 4, 376-86.

(1) The regeneration of the planarian Dendrocoelum lacteum has been tested at three different levels of amputation: just behind the eyes, half-way between the eyes and the pharynx, and just in front of the pharyngeal base. The planarians always regenerate in the first case, never in the last case, and about 7 times out of 10 in the intermediate case.

(2) Experiments involving amputations and irradiations show that there are regeneration cells (neoblasts) in the regions behind the pharyngeal base, regions which are unable to regenerate.

(3) When they are not irradiated, these neoblasts are able to move towards the head and to restore the irradiated regions. They reconstitute a blastema with eyes, the number of cases directly depending on the prepharyngeal level of the section.

(4) The histology confirms the experimental results and demonstrates the processes of regression, of necrosis, and then of colonization of the irradiated regions.

16. Lender, Th. & Gabriel, A. Étude histo-chimique des néoblastes de Dugesia lugubris (Turbellarié Triclade) avant et pendant la régénération. Bull Soc. Zool., France, 1960, 85, 100-110.

Biochemical study of the neoblasts of D. lugubris before and during regeneration show that alkaline phosphatase is not present at this level. However, sulfhydryl groups (-SH), bound to the proteins, are discernable in some regenerative cells in the planarians studied. The neoblasts can be readily identified by staining with methyl-pyronine: red nucleolus and cytoplasm (RNA) and green nucleus (DNA). This characteristic has allowed determination of the number of neoblasts in the intact, non-regenerating animal and during different stages of regeneration (from 24 hours to 6 days). In the whole flatworm, the neoblasts are distributed according to a decreasing anterior to posterior gradient. Examination of the neoblasts at various times led to the construction of curves showing variations in their distribution as a function of the level studied and of the stage of regeneration.

17. Lender, Th. L'Inhibition spécifique de la différenciation du cerveau des planaires d'eau douce en régénération. J. Embryol. exp. Morph., 1960, 8, part 3, 291-301.

(1) During regeneration of the head of the fresh-water planarians Dugesia lugubris and Polycelis nigra a brain inhibits the differentiation of another brain.

(2) When bearing a graft of a brain, a decapitated D. lugubris regenerates a head, but the regeneration of the brain is totally or partly inhibited.

(3) The inhibitory power of the brain is due to its secretion of chemical substances. These can be demonstrated in crude homogenates of heads and in the supernatant of centrifuged homogenates of heads. Homogenates of tails are inactive.

(4) The inhibitory substances are not species-specific. A homogenate of D. lugubris heads inhibits brain differentiation in P. nigra.

(5) The inhibitory substances are organ-specific. In the presence of either a brain graft or a homogenate of heads, a decapitated planarian regenerates all the head tissues except the brain.

(6) The antero-posterior diffusion gradient of organ-specific brain inhibitors corresponds to the morphogenetic gradient of Child.

18. Stéphan-Dubois, Françoise & Gilgenkrantz, Fernand, Régénération apres transplantation chez la Planaire Dendrocoelum lacteum. Comptes rendus des seances de la Société de Biologie, 1961, 155, 115.

The neoblasts in the posterior region are totally potential (this confirms the conclusions of 1959) but a cephalic blastema can only reconstitute itself by dividing a prepharyngeal area from a potential area. In the exact frame of experiments, which come to be described, each territory hence conserves its own potentials.

19. Wolff, Etienne. Migrations et contacts cellulaires dans la régénération. Experimental Cell Research, 1961, Suppl. 8, 246-259.

(1) Numerous animal species possess special cells, called cells of regeneration, which play a leading role in the process of reconstruction of a mutilated part.

(2) These cells of regeneration have properties in common. One of the most important is their capacity for migration. This property can be shown by the method of regional irradiation, by which healthy migratory cells are forced to cross a zone where all the cells are in the process of necrosis.

(3) the "neoblasts" of planarians are spread throughout the body in the middle of the parenchyma. They are able to flow from any region toward the mutilated part, crossing the entire body of the planarian to repair all tissues.

(4) In the limicolous Oligocheta the "neoblasts" are large cells, products of the activation and metamorphosis of special cells of the dissepiments. They migrate towards the amputated surface, crossing a number of segments which varies with the species. They reconstitute only mesodermal organs. The cells of regeneration of the ectoderm and of the endoderm effect only feeble displacements.

(5) In the Polycheta (Nereis) the cells of regeneration arise from leucocytes free in the coelomic cavity. After amputation they flock towards the sectioned surface; they may cross a dozen segments. They form a blastema of regeneration which is capable of regenerating all tissues.

(6) In the amphibian Urodeles (Triturus cristatus) the cells of regeneration of fibroblastic aspect accomplish migrations, important in absolute value, but relatively confined in accord with the total length of the limb (maximum extent of migration: 5mm). They are directed in simple or multiple flux toward the amputated surface. This flow can give birth to a blastema of regeneration which joins with the neighboring blastema. A strong polydactyl limb often results.

(7) The factors which determine the migration of cells of regeneration have been studied especially in planarians. The migration is launched by an amputation, or simply a downright wound. The movement is oriented in the direction of the wound, stopping at the source of the stimulus. This migration occurs throughout the body of the animal.

(8) In vitro explanation experiments tend to show that the attraction of neoblasts is determined by diffusible substances which emanate from the injured region.

20. Lender, Th. & Gabriel, A. Le comportement des neoblastes pendant la régénération de la planaire Dugesia lugubris. Bull. Soc. Zool. Fr., 1961, 86, 67-72.

(1) The neoblasts of fresh-water planarians are free cells in the parenchyma. A large nucleus with a nucleolus is surrounded by a thin layer of cytoplasm rich in RNA.

(2) During regeneration the neoblasts build a blastema in which the tissues become differentiated. Typical mitoses are visible in the neoblasts of the bud and the neoblasts distributed in the parenchyma.

(3) Neoblasts are abundant in the parenchyma of planarians basted for two months.

They are used to build the bud of regeneration.

(4) In planarians irradiated "in toto" with X-rays, the ability to regenerate disappears with the neoblasts, which entails the death of the animal.

(5) In planarians irradiated locally, behind the wound, the neoblasts of the irradiated zone disappear. But this zone is colorized by regeneration cells which build the blastema of regeneration.

21. Wolff, E. Recent researches on the regeneration of planarians. In Rudnick, D. (ed.) Regeneration, New York: Ronald Press, 1962.

The study of freshwater planarians endowed with the power of total regeneration has elucidated certain factors which play a role in morphogenesis during regeneration. Under certain experimental conditions (local irradiation, culture of regeneration blastemata) it is possible to separate almost completely the cells which are responsible for regeneration from other cells of the body. These cells are the neoblasts, the distribution and migrations of which have been analyzed. Endowed with potencies which are multiple and probably total, they form a regeneration blastema which can be considered as a graft of undifferentiated embryonic elements on the adult organism. The interactions between the regeneration blastema and the base consisting of the old tissues have been analyzed. The morphogenesis of regeneration can be explained by a succession of inductions and inhibitions. Phenomena of induction, comparable to those which intervene in amphibian morphogenesis, have been demonstrated in the regeneration of planarians. It can be assumed that the base induces the formation of an anterior blastema, in which the first differentiation is that of the brain. The brain has an inducing action, through the intermediary of diffusible substances, on the eyes. The differentiation of the head is thus achieved. It is supposed that the prepharyngeal region is induced by the head. But it is known that the pharyngeal region is induced by the prepharyngeal region. The pharynx differentiates spontaneously within this pharyngeal zone. Finally, the copulatory organs are induced by the zone of the genital glands which are anterior to them. Under usual circumstances, these mechanisms prevent, at all levels of the body, repetitive regeneration of an organ. The work of Lender has shown that there is a diffusible inhibiting substance in the brain. The strength of the action of this substance decreases with distance. Inhibitions are also exerted by the prepharyngeal and pharyngeal zones, but the existence of inhibiting substances is still hypothetical. Even though these results are as yet incomplete, it is felt that many phenomena can be explained by the hypothesis of inducing and inhibiting substances diffusing along the cephalo-caudal axis of the organism. The doctrine of physiological gradients would find a biological explanation and a concrete basis for further research.

22. Wolff, E., Lender, Th. & Ziller-Sengel, C. Le rôle de facteurs auto-inhibiteurs dans la régénération des planaires. Revue Suisse de Zool., 1964, 71, fasc. 1., 75-98.

(1) The inhibitory effect of organ extracts during the regeneration of fresh water planarians (Dugesia lugubris, Polycelis nigra, Dugesia tigrina) has been demonstrated for two regions of the body: the head and the pharyngeal region.

(2) Graft of brain, or head extracts prevent or delay the regeneration of the brain in an anterior head blastema. The supernatant of the centrifuged extracts has the same effect as the whole extract. The tail extracts do not delay the regeneration of the head.

(3) The supernatant of extracts of the pharyngeal region has a specific inhibitory effect on the regeneration of the pharynx, after this organ has been excised. Neither the head extracts, nor the tail extracts have such specific inhibitory action on the regeneration

of the pharynx; on the contrary, in some experiments, they have an entrancing action on this process.

(4) The authors formulate following hypothesis: specific inhibitory substances are produced by a regenerating organ or region, and prevent this organ or this region from being regenerated several times. Thus the regeneration is restricted to the reconstruction of the missing part.

(5) The regeneration of the missing parts of a planarian results from a balanced succession of inductions and inhibitions. In the case of the anterior regeneration, a sequence of inductions proceeding from the head towards the tail (brain, eyes, pharyngeal region, pharynx, copulatory apparatus) is controlled by antagonistic factors which are present in the remaining part and which act in the opposite direction.

(6) The theory of axial gradients (Child) and the concept of dominance can be explained by the presence of specific inhibitory substances which diffuse from a center where their concentration is maximum, to distant regions where their concentration is minimum.

23. Pentz, S. & Seilern-Aspang, F. Die Entstehung des augenmusters bei Polycelis nigra durch wechselwirkung zwischen dem augenhemmfeld und der augeninduktion durch das gehirn. Roux' Archiv fur Entwicklungsmechanik, 1961, 153, 75-92.

A. Prohibition zone of the eyes.

(1) The experiments were carried out with the paludical triclade P. nigra. This beast exhibits an eye line with a defined minimum interval between the eyes in the forebody along the dorsal line up to the root of the pharynx.

(2) Through extension of the tissue a moving apart of the eyes could be produced. Between the eyes that had been moved (pulled apart), new ones were induced.

(3) After development of a regeneration blastema the extended body line was pushed back to its normal size. The supernumerary eyes are therefore placed closer together than normally.

Through the eyes newly grown because of the extension there arises after the dissolution of the extension a significantly denser position of the eyes. Newly induced eyes, which were still in the growth process at the dissolution of extension, were hindered in their growth by the moving together of the eyes. However they continued the growth process at the renewal of extension.

(4) A reduction of the "supernumerary" eyes after dissolution of the extension could not be shown.

(5) The presence of a prohibition zone around every eye must be accepted.

(6) This prohibition zone has a radial spread. This could be proved by a swollen-like widening out of the edge of the eye. The eye-formation therefore resulted in a wider area.

(7) The prohibition zone of the eyes is responsible for the sequence of eye formation during normal regeneration.

B. Induction capacity of the eye rim.

(1) The induction capacity of the eyes (inductor = brain) is restricted to the lateral anterior body line up to the root of the pharynx. Through removal of the anterior eye-carrying part of the body eyes arise only in the regeneration blastema and not in the old tissues.

(2) Through a temporary experimentally produced prevention of the growth of the blastema (cramping of the tissues through a wedge-shaped cut) eyes are formed also in the uninducible edge. It appears that through the delay of the regeneration in the old tissues a head zone was built up temporarily, which evoked a new order of differentiation potential of the tissues.

(3) In view of the eye distances from one another and of the changing eye size, the eye pattern of the anterior body line is conceived as a result of the antagonism between the eye inducing effect of the brain and the prohibition fields of the eyes.

24. Hoff-Jorgensen, E., Løvtrup, Ebba, & Løvtrup, S. Changes in deoxyribonucleic acid and total nitrogen in planarian worms during starvation. J. Embryol. exp. Morph., 1953, 1, 161-165.

(1) The changes in total nitrogen and in deoxyribosides (DNA) have been followed during starvation of the planarian worm, Polycelis nigra.

(2) It was found that total nitrogen content after 60 days had been reduced to about 14 per cent of its initial value.

(3) It is not possible to exclude with certainty the occurrence of any DNA loss, but it is clear that the loss, if any, is much smaller than the loss in nitrogen.

(4) The implications of these results for previous morphological investigations of the effects of starvation on planarian worms are discussed.

25. Kanatani, H. Fission-promoting action of a water extract of planarian body in Dugesia gonocephala (Preliminary Report). Zool. Mag., 1964, 73, 52-7.

The effect of a planarian water extract on the occurrence of fission was studied in planarian, Dugesia gonocephala. Intact worms were cultured in a dilute extract of anterior body-halves. It was found that fission was greatly promoted in worms cultured in the water extract as compared with controls. The active principle, which was contained in the water extract, as found to be heat stable (100°C, 30 min.) and acetone insoluble. In order to determine the distribution of the active principle along the body axis, tests were made with water extracts from the head, middle and tail regions respectively. The greatest fission-promoting activity was found in the head extract; the extract of the middle region showed considerable activity. The tail extract had little effect, if any, on the occurrence of fission. The fission-promoting substance seems to decrease gradually in quantity from head to tail. The possible mechanism of the action of the fission-promoting substance is discussed in relation to head dominance.

26. Halas, E.S., Mulry, R.C. & Deboer, M. Some problems involved in conditioning planaria: electrical polarity. (See Reference # 6 under Learning and Other Behavior)

27. Barnes, C.D. & Katzung, B.G. Stimulus polarity and conditioning in planaria. (See Reference # 11 under Learning and Other Behavior)

28. Flickinger, R.A. A gradient of protein synthesis in planaria and reversal of axial polarity of regenerates. Growth, 1959, 23, 251-271.

Intact planaria exposed to $C^{14}O_2$ or glycine- $1-C^{14}$ show a cephalo-caudal axial gradient of incorporation into protein. This pattern appears to be maintained in regenerating worms.

When cut sections of worms are embedded in agar and the anterior ends are placed in chloramphenicol, the biological polarity is altered in a significant number of cases, producing bipolar or reversed polarity regenerates.

Both chloramphenicol or colcemide tend to obliterate the axial gradient of protein synthesis. The evidence suggests that reversal of a gradient of protein synthesis causes reversal of biological polarity in regenerating planaria.

29. Lindh, N. O. The mitotic activity during the early regeneration in Euplanaria polychroa. Arkiv. Zool., 1957, 10, 497-509.

In the planarian flatworm Euplanaria polychroa the mitotic activity has an even distribution before regeneration, but as soon as a part of an animal is forced to regenerate, a graded distribution appears both within the different longitudinal parts and along the old axis, if the number of cell divisions in the parts are compared. The greater mitotic activity occurs just behind the old mid-part, from which point the activity decreases cephalically and caudally. However, the activity in the old head is next to the mid-part maximum. From a mitotic point of view the gradient means that a fore-part blastema is more easily formed in the posterior part of the animal than in the anterior. On the contrary, a hind-part is more easily reconstructed the more cephalically it has to be created. In the formation of a blastema the mitotic activity has a rhythmic course, which consists of a slow oscillation upon which is layered a fast one. The resulting rhythm is longitudinally graded and is different in fore- and hind-part regeneration. The duration of the four separate mitotic stages also shows a periodic variation during the regeneration. The variation of mitotic spectrum as well as the rhythm in mitotic activity differs with the age of the animal.

The origin of blastema cells is shortly discussed.

30. Lindh, N. O. The metabolism of nucleic acids during regeneration in Euplanaria polychroa. Arkiv. Zool., 1956, 9, 421-450.

The reflections of regeneration are studied on regard to the biochemical balance between nitrogen, sugar, fat and nucleic acids, all of which are correlated with reduced weight.

Before regeneration the quantity of nitrogenous compounds, fat, sugar and nucleic acids follows special gradients along the longitudinal axis. This graded distribution is stable and when a new animal is regenerated from an old part, the distribution is leveled according to the origin.

During regeneration the animal loses and recovers in weight in a rhythmic fashion, which suggests a variation in density of the animal, an unbalanced metabolism of fat compared with constituents of greater density or a real loss and re-absorption of metabolites. The nitrogenous compounds vary broadly speaking in accordance with the course of loss of weight. The variation in amount of sugar and fat is synchronous with the energy requirement during the different stages of regeneration. The content of RNA, DNA and FNA (free nucleic acids) fluctuates according to the different decomposing and synthesizing stages of regeneration.

The wounding evokes the periodic variation and synchronizes all reactions to a fitted metabolic system. The reactions assume a peculiar step-like character throughout the animal, and for this reason regeneration does not affect only the blastema. However, sometimes the synchronism is absent during the first six to seven days of regeneration, which possibly indicates an inborn more or less synchronically metabolic rhythm.

The metabolic conversions do not have the same result in the blastema and the peripheral old part. The old part retains an old-adapted pattern of metabolism, but the blastema is an actually rejuvenated tissue-conglomerate. Rejuvenation in this case is associated with proportionally low content of nucleic acids and polynucleotides, which have a very low content of non-dissociating protamine. An old animal always has a greater content of polymerized compounds, containing large amounts of protamine, but small amounts of free nucleic acids (FNA), which are very pure.

The significance of gradients and rhythm is discussed.

31. Lindh, N. O. The nucleic acid composition and nucleotide content during regeneration in the flatworm Euplanaria polychroa. Arkiv f. Zool. 1957, 11, 153-166.

The qualitative composition of RNA and DNA is not uniform along the animal axis and varies during a regeneration. This latter variation changes from the one longitudinal level to the other.

In the case of DNA the qualitative variation is confined to a changing adenine: guanine (or thymine: cytosine) ratio; adenine to thymine and guanine to cytosine being constantly 1. The fluctuations are due to a varying adenine (thymine) content; the guanine (cytosine) content, seems to be more stable. The variation of DNA composition is not correlated to its varying content but follows the rate of cell multiplication. Vigorous mitotic activity is characterized by a high A:G ratio, and consequently a double-wounded part has greater A:G ratio than a single-wounded. On the contrary a low A:G ratio before a regeneration signified a high potency of increased rate of cell division. After the regeneration a low A:G ratio of the new units is a sign of rejuvenation.

The composition of RNA varies with its actual content, low content mainly means a proportional dominance of adenine over the other bases; guanine proportionally dominates over the pyrimidines. This composition indicates an action of ribonucleases during a period of decreasing RNA content. During a period of dominating RNA-synthesis the A:G is lowered as well as A:U and G:C. Consequently RNA varies its composition by the variation of guanine and pyrimidines. Double-wounding and consequent far-reaching protein re-arrangements always inflict fundamental qualitative changes in RNA. In spite of a low RNA content, the $(A+G)/(U+C)$ and A:G ratios are low during a double wound-repair. In this respect the A:G ratios of DNA and RNA are converse.

The qualitative variation of free nucleotides is great and also longitudinally graded. Only AMP, ADP and ATP seem to reflect the energy consumption. The great amounts of UMP and UDP increase during a period of cytolysis of old tissues and increased general cell activity. The adenylic acids accumulate before and during a period of differentiation. In the proportional changes of nucleotides, the variations are at first in accordance with a localized reaction pattern, but later the reactions depend on the number of wounds. At the end of regeneration there is a great accumulation of free nucleotides. The mutual relation of these in the different parts is, however, changed, which indicates that the newly restituted animals do not have a uniform chemical composition and identical biochemical reactions but diverge from each other according to their origin.

REPRODUCTION

1. Kenk, R. Sexual and asexual reproduction in Euplanaria tigrina. Biol. Bull., 1937, 73, 280-294.

(1) Euplanaria tigrina occurs in at least two physiological races which differ in the manner of reproduction: a sexual and an asexual race.

(2) The asexual race, according to observations covering several years, reproduces exclusively by fission. Temperature and nutrition control the rate of fission but do not induce sexuality.

(3) The sexual race periodically develops reproductive organs and lays cocoons. After the breeding period has ceased, the sex organs degenerate and fission may occur at high temperature. The individual animal can outlive several periods of sexual activity.

(4) In nature, all planarians that have alternating (sexual and asexual) reproduction, appear to propagate sexually during the colder season and asexually during the

warmer season of the year.

2. Kenk, R. Induction of sexuality in the asexual form of Dugesia tigrina. J. Exp. Zool., 1941, 87, 55-69.

(1) Dugesia tigrina occurs in two physiological races, a sexual and an asexual race.

(2) If the anterior third of a sexual individual and the posterior two-thirds of an asexual animal are united by grafting, testes and copulatory organs develop in the "asexual" part.

(3) The occurrence of genital organs in the asexual component is probably due to the passing into it of endocrine substances which otherwise are lacking in the asexual race. However, the possibility of a migration of "sexual" tissue (neoblasts) into the asexual posterior part is not overlooked.

3. Hyma n, L. H. Environmental control of sexual reproduction in a flatworm. Anat. Rec., 1941, 31, Suppl., 108.

The common planarian Dugesia tigrina (Planaria maculata) exists in nature as sexual and asexual stocks. The latter reproduce exclusively by fission and no means of inducing sexual reproduction in them by environmental changes has been discovered. The sexual stocks develop a reproductive system annually in spring and lay cocoons for about 3 months thereafter; the sexual system then degenerates and fission becomes the mode of reproduction. Fission may also occur during the sexual phase. Contrary to the opinion of other workers, the sexual stocks do not have an inherent sexual rhythm but the appearance of the reproductive system is controlled by temperature. Sexuality can be induced in sexual stocks at any season by lowering the temperature to 15° C. or lower for 2 weeks or less and then raising it to around 25°C. Indications of the male system can be seen within 2 or 3 days after the temperature rise and viable cocoons begin to be laid within a week. Some individuals are so sensitive to temperature change that exposure to low temperature for only 2 or 3 days suffices to induce sexual development. Sexuality can be repeatedly induced in the same worm but apparently not immediately after a period of sexual activity. Only worms of a sufficient size respond to temperature change but some worms of apparently adequate size also fail to respond. The development of the reproductive system may be partial and the condition attained shortly after temperature rise does not progress further.

4. Anderson, J. M. & Johann, J. C. Some aspects of reproductive biology in the fresh water triclad turbellarian, Cura foremanii. Biol. Bull., 1958, 115, 375-383.

A careful re-examination of the details of sexual reproduction in isolated individuals of the triclad species Cura foremanii has been carried out, designed particularly to determine whether this reproduction involves self-fertilization or simply the parthenogenetic development of unfertilized eggs. The following conclusions have been reached:

(1) Without apparent detriment to the species, sexual reproduction can continue for many generations, and evidently indefinitely, under conditions of isolation which prevent the occurrence of copulation between individuals. This is true of four different stocks of the species, of diverse geographical origin.

(2) All individuals examined during active reproduction showed masses of spermatozoa occupying the seminal receptacles through which the eggs must pass in moving from the ovaries to the genital atrium.

(3) Studies of the development of the reproductive systems in immature isolated worms of known ages reveal that spermatozoa are mature and have already migrated to the seminal receptacles as early as 35 days after the emergence of the individual from its cocoon.

(4) Under constant conditions, isolated individuals deposit their first cocoons some time between the 40th and the 86th day after emergence; thus, the eggs in even these first cocoons have always been exposed to spermatozoa in the seminal receptacles.

(5) During this exposure to spermatozoa, the eggs are penetrated by them; eggs have been observed in freshly deposited cocoons showing second polar bodies and vesicular female pronuclei, as well as distinctly staining sperm nuclei. Development of these self-fertilized eggs is presumably normal.

(6) It is thus clear that sexual reproduction in isolated individuals of Cura foremanii involves self-fertilization and not parthenogenesis.

One tantalizing question remains unanswered: whether copulation ever occurs in Cura, even among individuals in mass cultures. This species lacks a copulatory bursa, possessing only the bursa stalk which serves as a genito-intestinal canal. Copulation has not been observed during our experience with this species, and clearly from the standpoint of normal reproduction and development the process is completely superfluous. Breeding experiments with marked individuals might settle the question; until these can be devised, it seems probably that Cura foremanii may have dispensed with copulation as a feature of its sexual reproductive processes.

5. Jenkins, M. M. & Brown, H. P. Sexual activities and behavior in the planarian, Dugesia dorotocephala. Am. Zool., 1962, 2, 121.

Dugesia dorotocephala, commonly found in an asexual condition in springs and spring brooks throughout the United States, has been found in sexual form in a number of springs in Oklahoma in which a constant temperature is maintained throughout the year. In Buckhorn Springs, Murray County, numerous sexual animals and fresh cocoons have been observed every month in the year. Four generations from this source have been reared and studied in the laboratory. Laboratory reared D. dorotocephala attain sexual maturity approximately 6 months after hatching, and remain continuously sexual for a little over a year. During the months of sexual maturity, copulating pairs can be seen frequently in the cultures, particularly within the first hour after the worms are exposed to light. Positions assumed during copulation are extremely variable and rarely correspond to textbook diagrams. Only a few minutes is necessary for the complete act. Cocoon deposition requires 3 to 4 hours. Occasionally the contents of a freshly deposited cocoon are ingested by another planarian. During the months of sexual activity fission is an extreme rarity. As cocoon production wanes, fission products begin to appear. The mature worms, which show external signs of aging, decrease in number, the fate of the fission products has not yet been established.

6. Jenkins, M. M., & Brown, H. P. Cocoon-production in Dugesia dorotocephala (Woodworth) 1897. Trans. Amer. Microscop. Soc., 1963, 82, 167-177.

(1) A study was made of cocoon-production in planarians of the species Dugesia dorotocephala, obtained from springs in Oklahoma in which a constant temperature is maintained throughout the year. The studies included both worms of unknown age, collected from springs, and worms of known age, hatched and reared in the laboratory.

(2) Contrary to earlier reports for this species, the planarians studied produce cocoons the year around, both in the laboratory and in the natural habitat.

(3) Cocoon-production appears not to follow a season cycle, but to depend upon

the age of the animal. The planarians become sexually mature about six months after hatching, and are sexually productive for a little over a year. Fission then begins, and both worms and fission fragments begin to die.

(4) The period of incubation for cocoons of this species is three to four weeks at a temperature of 18.5 °C. The average number of young produced per cocoon is 16.5. The juveniles are somewhat pigmented at birth, and otherwise resemble adult planarians except that they have disproportionately large heads.

(5) Cocoon-deposition, and behavior in newly emerged juveniles are discussed.

TISSUE CULTURE

1. Murray, Margaret. The cultivation of planarian tissues in vitro. J. exp. Zool., 1927, 47, 467-505.

For planarian explants the optimum concentrations of Locke solution (diluted with well water, boiled and filtered) lie between 1/10 and 1/2 the concentration required by chick explants. Concentrations between 1/8 and 1/25 are tolerated. Intact worms, however, tolerate indefinitely all concentrations between well water and Locke solutions containing from 1/4 to 1/5 and salt-total of the vertebrate-isotonic solution. The organ of osmotic regulation is apparently the external worm.

In hanging-drop cultures the cells survive in fluid media as long as ten to fifteen days; they are pseudopodially active and show some cell division, but not sheet-like proliferation. When an agar substrate is afforded the explant, a conspicuous membrane-like outgrowth of tissue may be seen within four hours. This increases up to about twelve or sixteen hours.

Serum or tissue extracts of the following animals have been introduced into the culture media without injurious effect upon short-term explants from adult planarians--tapeworm, clam, snail, isopod, sheep. Sheep serum is stimulating. The artificial media used are Locke solutions, to which are added dextrose and peptone, glycogen, leucine, arginine, and tyrosine, respectively. Dextrose or glycogen prolongs the life of the cells considerably beyond that in a Locke solution alone. The mixture of dextrose and peptone stimulates them to migration and the appearance of division beyond that which occurs in the other solutions use. Sheep serum and dextrose, added to the Locke solution, have much the same effect as peptone and dextrose. The amino-acids used are not favorable, arginine distinctly toxic.

The various types of cells have been identified in culture and their behavior studied.

The size of a typical explant is about 1/120 (or less) the volume of a whole worm. When such pieces are introduced into hanging-drop conditions, a variable percentage of them may fail to spread out amorphously in the form of tissue cultures, and may round up to form individuals, some of them obviously polar and others such that no polarity is discernible. The polar individuals are the more viable, other things being equal. Physical conditions are of importance in the frequency of occurrences and the longevity of individuals under tissue-culture conditions. The physical conditions include size and depth of drop, nature of substrate, and osmotic pressure of solution. Apolar individuals may form originally from fragments of the explant, or secondarily from the debris produced by the disintegration of a polar individual.

2. Sengel, Catherine. Culture in vitro de blastèmes de régénération de planaires. J. embrajol. exp. Morph., 1960, 8, 468-476.

(1) Young regeneration blastemata of planarians have been cultivated in vitro on an agar-embryo extract culture medium which is a modification of the organ culture medium devised by Wolff & Haffen.

(2) Transverse cuts were made in whole worms and 3 days later the blastemata were isolated on the culture medium, where they survive for 10-15 days. There is no visible growth, but the observations reveal pigment formation by the third day, the appearance of muscles on the fourth or fifth day, and the presence of the eyes and brain by the tenth day. All three types of differentiation have been observed in the cultures of head blastemata, but only pigment and muscles are present in the cultures of tail blastemata.

(3) When two or more head blastemata are cultured together they differentiate into a larger head with supplementary eyes. The association of two or more tail-blastemata does not result in the differentiation of eyes or brain. The combination of one head blastema with one or two tail blastemata in such cultures gives rise to a small planarian with a pharynx and a gut.

(4) The method of culturing regeneration blastemata in vitro reveals that the fate of the blastemata is determined quite early. A head blastema can differentiate cephalic structures, whereas a tail blastema cannot. However, if a head blastema is associated with a tail blastema in culture, then they differentiate into a complete planarian.

3. Anserin, K.D. & Buchsbaum, R. Observations on planaria cells cultivated in solid and liquid media. J. exp. Zool., 1961, 146, 153-161.

(1) Tissues of the planarian flatworm, Dugesia tigrina, were cultivated in one type of solid medium and two types of liquid media. All the media were enriched with planarian tissue extract.

(2) Solid medium favored cell migration, and proved to be also adequate for regeneration and morphallaxis occurring inside the explants.

(3) Both types of liquid media inhibited cell migration.

(4) Liquid medium consisting of worm saline and planarian tissue extract was adequate for the formation and survival of so called "restitutional bodies" of Freisling and Reisinger ('58), and for survival and regeneration of larger pieces of tissues.

(5) Addition of chicken plasma to the liquid medium inhibited both the formation of restitutional bodies and the survival and regeneration of larger explants.

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In addition to the above listed references, the following articles reporting planarian research have appeared in the WORM RUNNER'S DIGEST:

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