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The Development of Acquired Immunity to Tapeworms and Progress Towards Active Immunization, with Special Reference to *Echinococcus* spp.

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An assessment is made of the present state of knowledge on acquired immune responses developed by the intermediate and definitive hosts to tapeworm infections. From this evaluation, some gaps in knowledge and some of the problems associated with the development of practical immunization techniques are described. The principal conclusion reached is that absolute resistance to the larval stage can be acquired and resistance to a number of cestode species can be artificially induced in a number of hosts. Thus, research directed towards isolation and characterization of the functional antigens may lead to the development of vaccines for use in public health programmes, such as for the control of echinococcosis, as well as for improving the present status of meat hygiene in regions where cysticercosis, for example, exists.

Innate immunity to tapeworm infections, in the absence of previous exposure, may result from a wide variety of, as yet, incompletely defined physiological, biochemical, nutritional and environmental factors.

By definition, acquired immunity involves a response by the reticulo-endothelial and lymphopoietic systems following an exposure to antigen, giving rise to an altered reactivity of marked specificity. Evidence for the involvement of these systems is most usually provided by passive transfer studies. Reinfection trials and vaccination studies, as well as observations on the development of resistance in the field, can only give supportive evidence.

The host may develop protective responses to at least three phases of the life-cycle of the tapeworm. For example, responses may occur to the embryo, to the strobilar stage and to the cyst or bladder stage of cyclophyllideans, or to the strobilar and the plerocercoid or sparganum stages of pseudophyllideans. The strobilate phase develops sexually, with few exceptions, strictly within the lumen of the intestine, but the embryo penetrates the mucous membrane of the intestine and develops into a cyst in the host tissues. Protection-inducing (PI) antigens, available during each phase of the life-cycle, may differ quantitatively and qualitatively; they have not been characterized and are therefore referred to as a complex.

In this review, which is confined to a consideration of host responses to tapeworms, an attempt is made to summarize significant research on the development of immunity by the intermediate and definitive hosts, and from this, to provide a consistent definition of some of the problems still to be solved before practical immunization procedures against, for example, *Echinococcus* spp., the etiological agents of hydatid disease in man, might be realized.

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METHODOLOGY

BIOLOGICAL SYSTEMS

In the family Taeniidae, vertebrates are host to both the cystic and strobilate phases. In almost all other families of cestodes, however, the strobilate phase develops in a vertebrate, while the cystic phase occurs in an arthropod. Most of the important human cestode pathogens are found within the family Taeniidae.

One of the important biological systems in which to study host defence mechanisms is the dwarf tapeworm of mouse and man, *Hymenolepis nana* (*H. nana* var. *fraterna*, Stiles). In the mouse or rat *H. nana* has the unique capacity to infect a vertebrate host directly from eggs or indirectly from cysticercoids developed in arthropods. Thus, it is capable of following the 2-host pattern typical of cyclophyllideans or of following a direct cycle in which the mouse or rat, depending on the strain, can act as both the definitive and the intermediate host.

For the study of immune mechanisms to the tissue phases of cestodes, the rabbit metacestode *Taenia pisiformis*, the rat metacestode *Hydatigera taeniaeformis* and the microtine rodent metacestode *Echinococcus multilocularis* may be used. The latter has a unique property in that it can be maintained in the laboratory by passage of the protoscolex from rodent to rodent without the intervention of a sexual phase (Norman & Kagan, 1961).

Host–cestode systems in large animals include *T. saginata* in cattle and *T. hydatigena*, *T. ovis* and *E. granulosus* in sheep. However, the study of these systems poses problems of maintaining statistical numbers of animals in worm-free environments.

For studies on immunity to the strobilate phase, the mouse or rat cestode *H. diminuta*, the cat cestode *H. taeniaeformis*, and the dog cestodes *T. hydatigena* and *E. granulosus*, are available. The latter species is particularly useful in that many worms can be established and, unlike the larger species of cestode, its growth is not related to the numbers established. Thus, immunity to both numbers and retardation of growth can be studied without the association of a so-called " overcrowding effect " (Gemmell, 1962a).

Several systems can be used for studies on cross-protection in definitive or intermediate hosts. These include *H. nana*, *H. diminuta* and *H. citelli* in mice, and *T. hydatigena*, *T. ovis* and *E. granulosus* in dogs and sheep. It is to be emphasized, however, that the strains used may differ antigenically from one laboratory to another.

INTERMEDIATE HOST

IMMUNITY ASSOCIATED WITH HUMORAL FACTORS

Serum transfer

Miller (1932e, 1934) and Miller & Gardiner (1932a, 1932b, 1934) first demonstrated humoral involvement in immunity to cestode infections by the successful passive transfer of serum from donor rats infected with, or immunized against, *H. taeniaeformis*. Serum collected from donors within 10 days of an infection conferred immunity and this was effective in arresting development of cysticerci, even when given to recipients up to 9 days after they had been dosed with eggs of *H. taeniaeformis*.

Although an unsuccessful attempt to demonstrate passive transfer of immunity to *T. saginata* using serum from naturally infected cattle in Kenya has been reported (Froyd, 1964a), laboratory trials confirming Miller's findings have been recorded for *T. pisiformis* in rabbits (Kerr, 1934, 1935), for *H. nana* in mice by Hearin (1941) and for *T. hydatigena* in sheep by Blundell, Gemmell & Macnamara (1968).

Maternal transfer

As well as by passive transfer in serum, immunity is also transmitted from the mother. Thus, the offspring of rats infected with *H. taeniaeformis* or immunized with tapeworm tissue also show enhanced resistance to infection (Miller, 1932c, 1935).

Larsh (1942, 1944, 1951), using *H. nana*, also demonstrated that the immunity of infected mothers was transferred to foetuses *in utero* and to new-born mice in milk. The protective effect transferred *in utero* was of shorter duration than that transmitted in milk. However, an active infection with *H. nana* in the mother, concomitant with pregnancy, was not found to be a prerequisite for transfer of immunity.

A degree of protection, less than that obtained following a natural infection, was transferred to offspring after the artificial immunization of mothers.

Conflicting reports, however, have been made by Sima (1937), who was unable to demonstrate parental transfer for *T. pisiformis* in rabbits, and by Urquhart (1961), who also was unable to show transfer of immunity in colostrum of cattle naturally infected with *T. saginata.*

Qualitative changes

Campbell (1938a, 1938b, 1938c), working with *T. pisiformis* in rabbits and with *H. taeniaeformis* in rats, detected humoral antibodies as early as 7 days after an oral infection or after artificial immunization. He observed that serum taken from donors during the second week of an infection exerted a protective effect in recipients primarily, but not exclusively, against the establishment of the challenge infection. Immune serum given 8 days before, and up to 2 days after, infection completely protected recipients. If immune serum was injected later than 10 days after infection it was not protective against this early phase. Absorption of such serum with tapeworm tissue *in vitro* removed part of the protective effect of the serum, particularly that directed against encystment.

In contrast, serum taken 4 weeks after artificial infection, but not after vaccination, was associated with a distinct effect, primarily against the destruction of larvae following their encystment at the site of election in the recipients. In this case, the protective effects were not neutralized by absorption of serum with strobilate tissue *in vitro.*

Campbell's valuable observations not only confirmed Miller's work, but demonstrated that the protective properties of immune serum appear to alter qualitatively during the course of an infection. The former he referred to as " early " and the latter as " late " immunity.

Sites of response

Campbell's work in demonstrating a response before encystment and another associated with immunity after encystment led to biological studies on the sites of the reaction. Leonard & Smith (1939) injected hatched oncospheres of *T. pisiformis* into the mesenteric vein of immune and non-immune rabbits. The numbers established were similar in both groups. Subsequently, Leonard & Leonard (1941) considered that immunity to cestode infections has two distinct phases: the first acting at the intestinal level and the second occurring in tissues against the growing metacestode.

In an attempt to circumvent a possible phase of immunity at the intestinal level, Froyd & Round (1960) superimposed infections of *T. saginata* in adult cattle, immune to an oral challenge, by a parenteral injection of artificially activated embryos. On being able to create a tissue infection, they suggested that the intestinal phase had been successfully by-passed. Other evidence for a response at the intestinal level has been presented by Bailey (1951), who found few *H. nana* larvae in histological sections of the intestinal wall of immune mice. Gemmell (1962b) suggested that the intestinal mucosa acts as a barrier, not only in acquired immunity, but also in natural resistance to some species of cestode.

With the exception of *H. nana,*[1] which may differ in several respects from other species, there is as yet no unequivocal evidence defining just where the first reaction occurs. In this review of acquired and induced immunity, Campbell's early response is for descriptive purposes referred to as " pre-encystment " and his late response as " post-encystment " immunity. It may well be, however, that the latter may consist of several distinct reactions. Humoral and cellular mechanisms involved in these reactions are discussed in a later section.

ACQUIRED RESISTANCE BY THE INTERMEDIATE HOST

Pre-encystment immunity

Resistance following a single exposure. Since the discovery of the direct life-cycle of *H. nana* (Grassi, 1887), there have been several noteworthy contributions on innate and acquired immunity by the intermediate host to reinfection and superinfection by this species; see, for example, Joyeaux (1920, 1925), Woodland (1924), Brumpt (1933), Shorb (1933), Hunninen (1935a, 1935b, 1936), Hearin (1941), Bailey (1951), Weinmann (1958, 1964, 1966), Heyneman (1961, 1962a, 1962b) and reviews by Larsh (1951) and Heyneman (1963).

Shorb (1933) made an extensive quantitative study of the factors related to host resistance. He interpreted breaks in the course of egg-production in naturally acquired infections of *H. nana* as indicating the loss of old infections and the development of new ones. Hunninen (1935b), however, found that, even with egg doses of 250–500 eggs, super-

[1] The site of election of *H. nana* is in the mucosa of the intestine, whereas that for most taeniids is in the liver, the lungs, or muscles or in all these sites.

infections were completely inhibited from the 5th day. Hearin (1941) reported that this resistance was still complete 163 days after the initial infection had been removed by anthelmintic treatment. Subsequent studies have confirmed that immunity can be acquired to this organism.

Further laboratory evidence from other cestodes, which supports the probability that a relatively high level of pre-encystment immunity is a characteristic response of the intermediate host to reinfection with tapeworms, includes studies on the development of acquired resistance to *H. taeniaeformis* in rats (Miller, 1931a, 1931b; Miller & Massie, 1932) and to *T. pisiformis* in rabbits (Kerr, 1935).

Studies with larger animals also support the probability that acquired immunity is a characteristic response of vertebrate hosts to tapeworm infections. For example, solid resistance was present to a superinfecting dose of 5000 eggs of *T. hydatigena* 7 weeks after the sheep had received a sensitizing dose of as few as 50 eggs (Sweatman, 1957). Soulsby (1963) was unable to superinfect calves 10 months after they had received a sensitizing dose of as few as 100 000 eggs when 4–6 months of age. A high level of resistance was recorded in lambs to a challenge dose of 7000 eggs of *E. granulosus* 10 months after the animal had received 50 000 or 100 000 eggs. When the sensitizing dose was less than 50 000 eggs, no immunity, or only a low level of immunity, was produced (Sweatman et. al., 1963).

Resistance following repeated exposures. All the studies with large animals mentioned above involve only a single sensitizing dose and various time intervals between sensitizing and challenge doses. The animals used were maintained under a " homologous " tapeworm-free environment. The duration of the pre-encystment phase of acquired immunity has not yet been well characterized. Field-observations and superinfection trials, in regions where animals are repeatedly exposed to small doses of eggs, support the probability that a life-long resistance to the pre-encystment phase, unassociated with an age-resistance factor *per se*, can be acquired; for example, by cattle to *T. saginata* (Penfold, Penfold & Phillips, 1936; Penfold & Penfold, 1937; Peel, 1953; Urquhart, 1961; Froyd, 1964b). Surveys suggest that a similar life-long resistance to *T. hydatigena* can be acquired by sheep (Gemmell, 1961). Although survey data for *E. granulosus* (Pullar & Marshall, 1958; Gemmell, 1961) suggest that the number of cysts in animals increases with the age of the animal, these data cannot be used to infer (Weinmann, 1966) that life-long resistance to *E. granulosus* does not develop in endemic regions. Cyst growth varies enormously (Gemmell, 1966a); small cysts may be overlooked and it is difficult, if not impossible, to distinguish an initial from superimposed natural infections.

Post-encystment phase

Most studies on acquired immunity to superinfection have been concerned with the more spectacular pre-encystment immunity. There is, however, no doubt that a great proportion of cysts which become established in the tissues of the host fail to survive indefinitely.

Penfold, Penfold & Phillips (1936), working in Australia, considered that the isolation of 1 viable cysticercus of *T. saginata* from 100 lesions was quite consistent with an infection of 11 weeks' standing. Penfold & Penfold (1937) found that in adult cattle given 400 000 eggs, cysts failed to survive for more than 9 months. McIntosh & Miller (1960) and Dewhurst, Cramer & Pistor (1963) observed viable cysticerci more than 1 year after cattle were fed eggs of *T. saginata.*

Urquhart (1961) conducted a thorough study of cysticercosis in Kenya. Here, calves are naturally infected at, or within a few weeks of, birth, and Urquhart found that cyst survival may be life-long in some animals. In comparing his results with the Australian experience, Urquhart suggested that the size of the initial dose of eggs, as well as the age of the animals at the time of initial infection, may influence the longevity of the cyst in this species. Soulsby (1963), working with calves in a " *T. saginata*-free " environment, recorded that a degree of immunological unresponsiveness could be induced, not only to the pre-encystment phase, but also to the post-encystment phase of *T. saginata*, if eggs are fed to calves at, or shortly after, birth.

Although factors responsible for survival of cysts have not yet been well defined, it is clear that acquired resistance is involved. Not all species, however, are destroyed as readily as *T. saginata.* For example, some cysts of *E. granulosus* can survive indefinitely in immunologically competent sheep when these are given an oral dose of eggs at 6 months of age. Within 30 months, however, cyst size and structure vary from that of a pin-head, in which parasitic material is hard to find, to a bladder containing 60 ml of fluid. Of the embryos which became established, about half failed to survive. Dead and viable cysts may be found in juxtaposition in the same organ (Gemmell, 1966a).

ACTIVE IMMUNIZATION OF THE INTERMEDIATE HOST

Killed tissue

Strobilate tissue. In a series of studies, Miller (1930, 1931b, 1931c, 1932b, 1932d) recorded that rats can be partially protected against a challenge infection of *H. taeniaeformis* with a series of intraperitoneal injections of crude homologous strobilate tissue homogenates or extracts. In most of the trials, a marked effect was induced against the pre-encystment phase, but in all trials the major effect was directed against the post-encystment phase. Campbell (1936) found that a series of intraperitoneal infections of adult or cyst material of *H. taeniaeformis*, or protein fractions of these, induced a protective response. Those protein extracts from fresh worm material produced a relatively strong effect against the pre-encystment phase and those from dried worm material produced an effect against the post-encystment phase. Larsh (1944) also obtained a high degree of protection against the pre-encystment phase of *H. nana* following repeated intraperitoneal infections of extracts or freshly macerated homogenates of strobilate tissue.

Miller & Kerr (1932) and Kerr (1934, 1935) obtained only a partial resistance to the pre-encystment phase of *T. pisiformis* in some animals following repeated injections of dried powdered or freshly macerated tapeworm tissue. The development of cysts was totally inhibited in a few immunized animals but, in most cases, the difference between growth in the test animals compared with growth that the controls was one of degree only.

Cystic tissue. Turner, Dennis & Berberian (1937), using a series of injections of dried protoscolices and germinal membrane of hydatid cysts, failed to induce any response to the pre-encystment phase of *E. granulosus* in sheep. A marked protection, however, developed to the post-encystment phase, resulting in a reduction in growth with caseation and calcification of cysts, particularly of those deeply embedded in the tissues.

Eggs. No significant protection could be induced to the pre- or post-encystment phases of *T. hydatigena* in sheep following a single injection of up to 50 000 eggs killed at –70°C (Gemmell, 1964a).

Cyst fluid. Although hydatid cyst fluid is diagnostically antigenic for various immunodiagnostic tests, Dévé (1927, 1933, 1934a, 1934b) was unable to demonstrate any protective effect of cyst fluid or " hydatid sand " or membranes against a subcutaneous challenge infection of protoscolices in rodents. Moya & Blood (1964) also reported that hydatid cyst fluid has no protective properties against a challenge infection of *E. granulosus* in sheep.

Viable tissue

Strobilate tissue. One of the first trials to determine whether viable strobilate material could induce protection was that described by Miller (1932d). He injected sections of living strobilar of *T. taeniaeformis* into the abdominal cavity of rats, and these animals were subsequently given an oral challenge with the eggs. This strobilar material brought about complete protection in almost all rats to the pre-encystment stage. Although Larsh (1944) demonstrated an immune response following the injection of killed tissues, Hearin (1941) was unable to demonstrate that any protection developed to *H. nana* following an intraperitoneal implantation of living homologous strobilar tissue.

Eggs and embryos. In a series of studies, Gemmell (1964a, 1965a, 1965b, 1966a) found that a single injection of homologous viable eggs or artificially activated embryos protected 3-month-old animals against oral challenge infections with *T. hydatigena*, *T. ovis*, *T. pisiformis* and *E. granulosus*. The total response (i.e., inclusive of the pre- and post-encystment phases) was absolute in almost all animals. The longevity of this response without revaccination has not yet been determined. Soulsby (unpublished data) using unhatched eggs of *T. saginata*, was able to induce a significant level of protection to a challenge infection in cattle maintained in an environment free from *T. saginata*.

Froyd (1961) was unable to protect calves with an injection of activated embryos of *T. saginata*. However, this does not constitute contrary evidence, since the animals were no more than 6 days old when they were vaccinated and Soulsby (1963) subsequently showed that tolerance to this parasite may develop if eggs are fed within a few days of birth.

Cross-protection

Miller (1932d) showed that protection against *H. taeniaeformis* does not develop in rats following injections of killed heterologous strobilate tissue, but that partial resistance to this species could be induced by the implantation of viable strobilate tissue of *T. pisiformis*. On the other hand, oral feeding of eggs of *T. pisiformis* failed to induce protection against *H. taeniaeformis*.

The embryos of *T. pisiformis* do not appear to share PI antigens with those of the sheep metacestodes, *T. hydatigena*, *T. ovis* or *E. granulosus*. Some cross-protection is, however, demonstrable between *T. hydatigena* and *T. ovis* to both the post- and the pre-encystment phases. The activated embryos, but not the unhatched eggs of these two species, protect sheep only against the post-encystment phase of *E. granulosus* (Gemmell, 1964b, 1967). *Echinococcus granulosus*, however, does not protect microtine rodents from *E. multilocularis* (Rausch & Gemmell, unpublished data). These observations suggest that the degree of PI antigen sharing between species is not necessarily associated with the generic relationships of the parasites, but seems, rather, to be associated with the closeness of the relationships between the parasitized hosts. These differences could be accounted for by the failure of the parasite to survive long enough in the abnormal host to induce the formation of appropriate antigens.

DEFINITIVE HOST

ACQUIRED RESISTANCE BY THE DEFINITIVE HOST

From a single exposure of lumen-dwelling worms

Most early studies on acquired resistance to lumen-dwelling forms involved few animals and, usually a single exposure. Miller (1932a) showed that from 1 to 5 additional *H. taeniaeformis* adults could be superimposed in cats already harbouring 1 or more. Palais (1934) failed to produce superinfections in 3 rats to *H. diminuta*. Seddon (1931) failed to reinfect 4 sheep with *Moniezia expansa* after the initial infections had been lost under conditions in which control animals became infected. Other trials (e.g., those of Joyeaux & Baer, 1936, 1938) using ligulid plerocercoids indicated that some immunity was present for 14 days after the removal of worms from ducks and herring gulls, but after 20 days the birds were readily reinfected. Other observations involving the use of *Railletina cesticellus* in chickens (Luttermoser, 1938), *T. hydatigena* in dogs (Vukovic, 1949) and *H. nana* in mice (Hearin, 1941) also gave uncertain or statistically non-significant results.

Chandler (1939, 1940a, 1940b), in a series of carefully designed experiments using a single superinfection or reinfection of several *H. diminuta* in rats, concluded that overcrowding and competition between worms together with factors involved in innate immunity may account for any apparent acquired immunity. He also inferred, however, that insufficient evidence had as yet (1939) been collected to exclude the possibility of acquired immunity to lumen-dwelling worms.

From an exposure of tissue-invading embryos

Since the description of the indirect cycle of *H. nana* in fleas, beetles and moths by Bacigalupo (1928, 1931, 1932) and in the large grain beetle (*Tenebrio molitor*) by Bailey (1947), important contributions towards an understanding of acquired immunity to lumen-dwelling forms have been made (Bailey, 1950, 1951; Heyneman, 1961, 1962a, 1962c, 1963; Weinmann, 1966).

Using this biological system, it has been possible to obtain quantitative and qualitative data on the immunogenicity between lumen- and tissue- dwelling forms. Heyneman's contributions can be summarized as follows:

(1) Resistance *per se* to the lumen-dwelling form of *H. nana* is not acquired following a single exposure to the lumen-dwelling form.

(2) A partial resistance to the lumen-dwelling form develops in animals previously exposed to the tissue-dwelling form.

(3) A significant resistance to the tissue-dwelling form develops in animals previously exposed to the lumen-dwelling form.

(4) Autoinfection may occur only when the lumen-dwelling form is introduced before the tissue form.

(5) A single exposure to eggs of *H. nana* (tissue phase) induces protection against a subsequent *H. diminuta* infection, even when the *H. nana* infection has been lost.

Weinmann (1966) similarly showed that following the removal of the worms resulting from a single exposure to 10 cysticercoids of *H. citelli*, mice were resistant for 2 weeks, but not for 6 months, to *H. citelli*. They were still partially resistant to the tissue form of *H. nana* during this period.

Thus, the major observations from these studies imply that the lumen-dwelling form is immunogenic against the tissue-invading form and vice versa, but a single exposure of the former gives no obvious protection against itself.

From repeated exposures of lumen-dwelling worms

The above-mentioned studies have been concerned with the effect of a single sensitizing exposure on the development of acquired resistance to lumen-dwelling worms.

Weinmann (1966) records that infections of *H. diminuta* can be superimposed one on another, without effecting the longevity of the worm burden, during the first month of infection. He was, however, unable to establish this cestode in mice about 4 weeks after 3 sensitizing doses with single cysticercoids had been given at 10-day intervals. He also demonstrated an enhanced resistance to the lumen- and tissue-dwelling forms of *H. nana* so treated.

Gemmell (unpublished data), working with *E. granulosus* and using statistically significant numbers of dogs, found that worm counts and growth rates of worms cannot be predicted for the first 5 infections but, thereafter, resistance, as manifest by a reduction of numbers of worms or limited growth, or both of these, can be acquired. This acquired resistance is not always absolute in all animals and it can be lost.

Field-evidence also supports the laboratory findings that resistance to the lumen-dwelling form can be acquired after repeated exposure. Apart from Seddon's (1931) and Stoll's (1935) observations on *M. expansa*, Boughton (1932) also found evidence for the development of resistance to another anoplocephalid (*Cittotaenia*) and Gemmell (1959) was unable to detect heavy infections in adult dogs maintained under the same conditions as younger dogs which were frequently found with heavy infections of *E. granulosus*.

ACTIVE IMMUNIZATION OF THE DEFINITIVE HOST

Killed tissue

Chandler (1940) was unable to detect any response to *H. diminuta* in rats following a series of subcutaneous injections of saline suspensions of dried powdered whole worms. Ohira (1935), however, partially protected cats against *H. taeniaeformis* by injections of homogenates of cyst membranes of this organism. Noda [1] also reported that artificial immunization with antigen from adults proved to be highly protective against *H. nana*.

Turner, Berberian & Dennis (1933, 1936) and Turner, Dennis & Berberian (1935) concluded, from a series of trials, that partial protection against *E. granulosus* can be induced by a series of injections of dried powdered germinal membranes and scolices. Similar results on a smaller scale, using killed germinal membranes and protoscolices, have been reported by Matov & Vasilev (1955) and Forsek & Rukavina (1959). In an attempt to confirm the results of these workers, Gemmell (1962) also found that a partial resistance can be elicited, particularly to the growth of the organisms; a somewhat more effective response was achieved using killed strobilate tissue rather than metacestode tissue.

Viable tissue

Heyneman's (1963) and Weinmann's (1966) observations (see above) on the antigenic relationships between the tissue-dwelling form of *H. nana* and the lumen-dwelling forms of *H. nana*, *H. citelli* and *H. diminuta*, appear to be very similar to those when the embryos of certain species are used to protect the definitive host against *E. granulosus*.

Preliminary observations suggest that intravenous or intramuscular injections of artificially activated embryos of the sheep metacestodes *E. granulosus*, *T. hydatigena* and *T. ovis* appear to give a degree of protection against *E. granulosus* in dogs. The protection so induced is not absolute in all dogs and is not stimulated by the lumen-dwelling challenge infection. It can, however, be restimulated by further injections of activated embryos (Gemmell, unpublished data). No such protection can be observed after injection of embryos of the sheep metacestode *Multiceps multiceps* or the rabbit metacestodes *T. pisiformis* or *M. serialis*. As might also be expected (see above), the embryos of the sheep metacestode *T. hydatigena* do not protect dogs against the rodent metacestode *E. multilocularis* (Rausch & Gemmell, unpublished data).

MECHANISMS

HUMORAL AND CELLULAR FUNCTION

Pre-encystment phase

Role of antibody. Hearin (1941) first observed (for *H. nana* in mice) that acquired resistance to the invading organisms can be detected within 9 hours and may be absolute within 24 hours, and it is still demonstrable 163 days later. This phenomenon was confirmed by Bailey (1951), Weinmann (1958) and Heyneman (1962b), although in their trials absolute resistance was not achieved within 24 hours.

[1] Cited in Heyneman (1962b).

Bailey suggested that the initial response is mechanical and operates at the epithelial lining, causing cells to become resistant to the invading organisms. Heyneman, however, considered the reaction involves a rapidly mobilized local concentration of cellular antibody or a cellular activation in the vicinity of the parasite activity, rather than a slower-acting serum antibody, not detectable in the circulation until a week or so after immunization.

Although local production of antibody cannot be discounted, a systemic response to account for the rapid reaction has so far not received serious attention. In this respect, Coleman & de Sa (1964) have shown a very rapid antibody response by mice to adult *H. nana* implanted in the intestine. The factor responsible for Hearin's observation and Campbell's (1938a, 1938b, 1938c) successful serum-transfer studies may perhaps differ only in the avidity, or perhaps the class, of the immunoglobulin elicited; this requires further investigation.

Specificity of the complex. Cross-protection studies (Gemmell, 1964b, 1967) suggest that the PI antigen complex is present in the eggs and embryos of the homologous, but rarely the heterologous, species. The evidence from most of the immunization studies (see above) suggests that the complex is also present in small amounts in the viable and killed homologous strobilar tissue, but, at least in *E. granulosus*, it does not appear to be available in cyst tissue (Turner, Dennis & Berberian, 1937) or in cyst fluid (Moya & Blood, 1964).

In vitro *reactions.* Silverman (1955, 1965) observed the deposition of precipitates around the so-called " penetrating gland " of the embryo placed in immune serum. He claimed that this was a reaction between antibody and the penetrating-gland secretions of the embryo. Lysis of the parasite could be envisaged if the organism were to be sensitized by antibody and if complement was fixed. This may turn out to be an oversimplification, since it is not yet clear whether *in vitro* results simulate *in vivo* conditions, or if metabolic products are, in fact, involved in the protective response.

Post-encystment phase

Role of antibody. The development of qualitative differences in the protective properties of serum during the course of an infection was first observed during passive transfer studies by Campbell (1938a, 1938b, 1938c). Quantitative changes in antibody titres or in serum proteins have also been observed during the course of an infection, for example, by Soulsby (1962) for *T. saginata* and by Sweatman et al. (1963) for *E. granulosus*. The relationship of these changes to the protective response has not been established.

The evidence from the active immunization studies (see above) suggests that the PI complex is present in the homologous killed or viable strobilar tissue, as well as, in *E. granulosus* for example, in killed cyst tissue (Turner, Dennis & Berberian, 1937) but not in cyst fluid (Moya & Blood, 1964).

Cross-protection studies (Gemmell, 1964b, 1967), using eggs or activated embryos of four species of taeniid tapeworms, indicate that the PI complex associated with the post-encystment phase is not the same as that associated with the pre-encystment phase of immunity. For *E. granulosus*, the post-encystment PI complex is present in the activated embryo, but not in the viable eggs, of a number of heterologous species. It may well be, however, that the complex is secreted after the period of activation, perhaps during the early stages of cyst development.

Tissue reactions. Leonard (1940) observed an accelerated tissue response in immunized rabbits. A similar response to *H. nana* was observed in immune mice (Bailey, 1951). Monkeys immune to *Spirometra mansonoides* were found to surround the spargana with tough cysts (Mueller & Chapman, 1937). Silverman & Hulland (1961) observed that the rate of development of *T. saginata* in naturally infected cattle was related to the intensity of the tissue reaction. The greater the response, the less well developed were the cysts, compared with cysts of similar age in the same animal. They suggested that a lytic zone may occur around the developing cysts and that the cuticle was in some way softened, prior to the invasion of leucocytes. They also suggested that this was achieved by an opsonin-like substance or by antienzymes and that the ultimate lethal effect was non-specific.

In vitro *reactions. In vitro* studies using the cysts of *H. nana* (Heyneman & Welsh, 1959) or the spargana of *S. mansonoides* (Mueller, 1961) and the protoscolices of *E. granulosus* (Šul'c & Ismagolova, 1962) in homologous immune serum have also revealed the formation of a precipitate.

Although, as with the pre-encystment phase, there is reason to believe that antibodies are involved in the protective response, it is again not clear whether *in vitro* results and *in vivo* mechanisms are comparable, or what part antibody plays in the lethal reaction that leads to the sterility or death of the developing cyst. For *E. granulosus*, the age of the animal to which

an infection is given may influence the subsequent development of protoscolices (Nosik, 1954). Similarly, Urquhart (1961) reported the possibility of the survival of cysts of *T. saginata* in some animals which become infected shortly after birth. Soulsby (1963) observed a degree of immunological unresponsiveness in calves to *T. saginata* when infected at birth. Schwabe, Shinaza & Kilejian (1959) reported an age-resistance factor to secondary infections of *E. granulosus* in white mice. The difference in susceptibility was related to the magnitude of the host's cellular response. Whether these age-resistance phenomena also involve a differential effectiveness of a humoral or cellular response has not yet been determined.

Within the intestinal lumen

Role of antibody. Although no humoral transfer studies have been reported, the results for a limited number of species (see above) suggest that resistance to the lumen-dwelling forms can be acquired and can be artificially induced.

Evidence is also reported that implies that responses can be induced against both the number of worms and their growth. Whether these effects are part of the same reaction, varying only in the degree of the response, or whether separate systems are involved, is, as yet, unknown.

From a limited number of active immunization studies using, for example, *E. granulosus* in dogs, it appears that the PI complex is present in viable embryos and in killed strobilar and cyst tissue.

In vitro *reactions.* A secretion has been observed at the anterior tip of the rostellum in 35-day-old *E. granulosus* (Smyth, 1963). The association between this secretion and Šul'c & Ismagilova's observations on the protoscolex has not yet been determined. Heyneman & Welsh (1959) observed precipitates and a change in the mobility of adult *H. nana* when they were cultured in serum from artificially immunized rabbits. Weinmann (1966) was unable to demonstrate precipitates in adult *H. nana* cultured in serum from naturally infected mice, but demonstrated a lethal effect on adults when they were cultured in mucosal extracts from the same mice.

Just what part humoral factors play in the rejection of, or interference with, the growth of lumen-dwelling forms, remains to be determined.

CONCLUSIONS

Immunity to metacestode infections can be demonstrated by passive transfer indicating involvement of the reticulo-endothelial system. Tapeworms, particularly the taeniids and hymenolepids, are nearly ideal biological tools for studies on resistance to metazoa, because absolute immunity can be induced in some instances.

Active immunization of some host species can be successfully carried out, using both killed and viable worm tissue, against a number of species of cestode. There seems to be a PI antigen complex associated with each phase of the life-cycle, and this appears to be available in the tissues in varying amounts during different phases of the life-cycle. There may also be species variation.

All these studies, however, have been made with crude materials. Research should now be directed systematically towards:

(1) Axenic culture of all stages of the life-cycle;

(2) The use of modern physico-chemical techniques to extract, fractionate and characterize the antigens;

(3) The definition of the role of each of these purified antigens in protective immunity;

(4) The characterization of the immune response and its interaction with the parasite.

Studies of this kind should lead to a better understanding of the antigen–antibody reaction and its effect upon the host–parasite relationship in metazoan disease.

RÉSUMÉ

Les auteurs ont passé en revue un choix important de publications dans le but de déterminer l'état actuel des connaissances relatives à l'immunité acquise ou artificiellement conférée à l'égard des cestodes. Ce travail les a conduits à délimiter certains domaines de recherche dont l'exploration devrait permettre la mise au point de techniques d'immunisation pour la lutte contre l'hydatidose et la cysticercose chez les animaux domestiques.

Un certain nombre d'études portant sur plusieurs espèces de cestodes ont montré qu'au stade larvaire il existe au moins deux réactions distinctes qui entraînent le rejet ou la mort du kyste. Chez les animaux qui présentent une immunité à l'égard du stade précoce ou de « pré-enkystement », l'infection est éliminée sans qu'il en reste de trace. Les données indirectes incitent à croire que l'une des réactions létales se produit pendant la période où l'embryon essaie de traverser la muqueuse intestinale. Chez les animaux qui présentent une immunité à l'égard du stade tardif ou de « post-enkystement », les kystes sont détruits *in situ* et les débris parasitaires peuvent persister indéfiniment sous forme de lésions fibreuses ou calcifiées.

On a démontré, pour plusieurs espèces, que l'immunité pouvait être artificiellement conférée à l'égard des deux stades de l'évolution et que l'embryon viable constituait une source importante de matériel antigénique. Certains des antigènes actifs paraissent communs à toutes les espèces, tandis que d'autres sont spécifiques de l'espèce.

Bien que les travaux sur l'immunité acquise ou induite artificiellement à l'égard du stade strobilaire aient été moins poussés que ceux consacrés au stade larvaire, il n'en ressort pas moins que, à l'égard de certains organismes, un faible degré d'immunité peut être acquis et conféré artificiellement.

Dans leurs conclusions, les auteurs proposent que les recherches soient maintenant orientées vers l'isolement des antigènes et la détermination de leurs caractéristiques. Ce type de travaux permettra, en effet, d'acquérir une meilleure connaissance de la relation hôte-parasite dans les infections à métazoaires.

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