Immunology of schistosomiasis *

This Memorandum, after summarizing the life cycle of the different species of human schistosome, reviews the present knowledge of the immunology of schistosomiasis. Each stage of the parasite contains antigen that may stimulate an immune response. However, at the moment there are no accepted serological in vitro *tests that correlate with protection; this develops only after the host has experienced a living infection, which suggests that the stimulation of immunity is due to some metabolic process involving the release of protective antigen. The adult worm, however, seems to be able to escape the immune mechanism of the host. Specific antigens are also released by the eggs, and the immune response against these antigens seems to cause granuloma formation around the egg itself. The granuloma is the main lesion found in schistosomiasis. Evidence for protective immunity in experimental animals and man is reviewed, together with the possible mechanism by which the adult worm escapes the immune response of the host. A review of methods used for the diagnosis of schistosomiasis and a list of recommendations for further research are also included.*

In recent years great progress has been made in the understanding of the basic immunological mechanisms operating in several pathological conditions. Schistosomiasis remains one of the major diseases of public health importance and it was considered useful to review present knowledge of the immunology of this condition as a first step in the planning of future research—which, it is hoped, may lead to improved immunodiagnostic techniques, a better understanding of pathogenetic mechanisms, and advances in control by immunological means.

LIFE CYCLE AND DESCRIPTION OF THE PARASITE

LIFE CYCLE

The schistosomes or blood flukes that infect man are digenetic trematodes belonging to the superfamily Schistosomatoidea; they have no muscular pharynx and they produce nonoperculated eggs. The three species of schistosome that commonly affect man have similar life cycles and develop by a succession of stages: egg, miracidium, first-stage sporocyst, second-stage sporocyst, cercaria, schistosomulum, and adult. The basic life cycle has an alternation of generations, with the sexual generation of adult schistosomes in the definitive vertebrate host and an asexual multiplicative stage in a molluscan host. A relatively short-lived, free-swimming stage, the miracidium, hatches from the egg and is infective to the snail intermediate host. In the appropriate snail host the miracidium becomes a first-stage mother sporocyst; this gives rise to second-stage daughter sporocysts that in turn produce numerous free-swimming cercariae infective to the vertebrate host. The cercariae usually enter through the skin and, after penetration, are known as schistosomula; these migrate and develop into mature, adult schistosome worms.

Although the species of schistosomes that infect man are similar in their basic life cycles, they differ in the morphology of the adults and in the shape of the eggs and of the larvae that hatch from the eggs. They also show marked differences in their infectivity to the particular groups of snails that they use as intermediate hosts and in their infectivity to other mammalian hosts. The adult schistosomes are an unusual group of trematodes in that they are

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elongated and superficially resemble roundworms. This is an adaptation to living inside blood vessels. They are also normally dioecious, although hermaphroditism has been reported. The female worm is held in the " schist " or gynaecophoric canal of the male during copulation and oviposition, and both sexes have oral and ventral suckers.

IMPORTANT SPECIES AND THEIR GEOGRAPHICAL DISTRIBUTION

Schistosomes infecting man

Schistosoma mansoni (Sambon, 1907). This species, which causes intestinal schistosomiasis, is transmitted by aquatic snails of the genus *Biomphalaria.* The mature adults usually inhabit the inferior mesenteric vein and its tributaries, and lay eggs with a lateral spine. It occurs in most African countries, parts of Arabia, in northern and eastern parts of South America, and in some Caribbean islands.

S. haematobium (Bilharz, 1852). This species causes urinary or vesical schistosomiasis. The adults inhabit mainly the veins of the vesical plexus and lay eggs with a terminal spine. It is transmitted by aquatic snails of the genus *Bulinus,* and is present in most African and in some middle-eastern countries.

S. japonicum (Katsurada, 1904). This species is usually transmitted by amphibious snails of the genus *Oncomelania* and the adults inhabit primarily the superior mesenteric vein and its tributaries. It occurs in the Philippines, Japan, and the Chinese mainland. Small foci have been found recently in Thailand, the Mekong catchment area, and Indonesia. A zoophilic strain in the province of Taiwan does not appear to develop to maturity in man and is therefore apparently nonpathogenic to man. The eggs have a small, vestigial spine.

Veterinary schistosomes

S. bovis (Sonsino, 1876). The adults inhabit the mesenteric portal system of cattle and sheep. It is transmitted by snails of the genus *Bulinus.* It occurs in Sardinia, Iraq, and parts of Africa, but its economic importance is not fully understood. The eggs have a terminal spine. The parasite is related to *S. haematobium.*

S. mattheei (Veglia & Le Roux, 1929). This species replaces *S. bovis,* to which it is closely related, in southern Africa. It infects cattle, sheep, and a number of wild ruminants. It can infect man, but it is usually only found in mixed infections with other schistosomes. It has been reported to cause a significant number of deaths among sheep in South Africa.

S. intercalatum (Fisher, 1934). This species is closely related to *S. mattheei* and infects sheep, goats, and *Hybomis* rats. It occurs in Zaire, Rwanda, Burundi, and Cameroon where many cases of infection in man, apparently of low pathogenicity, have been reported.

Avian and other schistosomes

Several schistosomes of birds and mammals (e.g., *Trichobilharzia ocellata* (La Valette), *Gigantobilharzia, Ornithobilharzia,* and *S. bovis*) may cause cercarial dermatitis in man. Cercariae of other mammalian schistosomes, such as *S. margrebowiei* and *S. rhodhaini,* may also penetrate man. Exposure of man to such schistosomes undoubtedly occurs in some areas of endemic schistosomiasis where it confuses serodiagnostic techniques, although it may possibly also confer some degree of heterologous immunity.

STAGES OF THE LIFE CYCLE

Adult worms

The dorsoventrally flattened, elongated males are shorter than the filiform females. The lateral flaps of the male fold together, possibly locked by minute spines in some species, to form the gynaecophoric canal or " schist " within which the female is held. Such paired worms are not necessarily *in copula* and oviposition is possible. The general shape is clearly adapted to life in blood vessels, and the well developed ventral sucker of the male anchors the worms against the blood flow.

Body orifices include a mouth that perforates the oral sucker, a gonopore slightly posterior to the ventral sucker, and a posterodorsal excretory pore. The integument is covered with spines, tubercles, and/or hairs. Recent electron microscope studies have shown that the integument of *S. mansoni* comprises 5–7 layers and that the outermost of these are being continuously regenerated. Unlike the teguments of some other types of parasite (e.g., nematodes) that of the schistosome is a living tissue rather than a dead, protective sheath.

Internally, circular and longitudinal muscles and other specialized muscles are present, and a network of mesenchymal cells surrounds the internal organs. The digestive system consists of a short oesophagus

leading to the intestine that divides in front of the ventral sucker and reunites behind the gonads to continue as a blind, posterior gut caecum. The contents, especially in the females, are usually black and contain pigment (possibly haematin) derived from the blood. The excretory/water balance system is made up of flame cells, collecting tubules, and an excretory bladder with a terminal pore. In the male the testes are arranged in one or two rows behind the ventral sucker. In the female a single, pyramidal ovary lies medianly in the transverse midbody line anterior to the seminal receptacle, and posterior to the ootype from which the single, straight uterus leads to the gonopore.

The adult worms are approximately 10–20 mm long. Detailed measurements and points of contrast for the adults and other stages of the three human schistosomes are given in Table 1.

Each worm pair produces 300–3 000 eggs per day, depending on the species, and adult worms may live for as long as 20–30 years, although the mean life span is probably much shorter (3–8 years). Carbo-

Table 1. Comparison and dimensions of the three major human schistosomes

	S. mansoni	*S. japonicum*	*S. haematobium*
Adult male			
length, mm	6-12	12-20	10-14
breadth, mm	2.00	0.50-0.55	0.75-1.00
No. of testes	4-13	6-9	4-5
cuticle	tuberculate	non-tuberculate	finely tuberculate
posterior union of gut caecae	anterior to midbody	behind midbody	about midbody
Adult female			
length, mm	7-17	16-28	16-20
breadth, mm	1.00	0.30	0.25
No. of eggs in uterus	usually 1	50 or more	10-100
position of ovary	anterior to midbody	about midbody	behind midbody
Egg			
length, mm	0.112-0.175	0.070-0.100	0.083-0.187
breadth, mm	0.045-0.070	0.050-0.065	0.040-0.070
spine	large, lateral	minute, lateral	moderate, terminal
passed in	faeces	faeces	urine
Miracidium			
length, mm	0.160	(proportionately smaller or larger than *S. mansoni*	
breadth, mm	0.062	(proportionately smaller or larger than *S. mansoni*	
Cercaria			
length of head, mm	0.100-0.150 in all species		
breadth of head, mm	0.030-0.060 in all species		
length of tail, mm	about 0.300 including furcae in all species		
No. of pairs of flame cells	4	3	4
Schistosomulum			
size at penetration, mm	about 0.100-0.120 × 0.030 in all species		
size in lung, mm	about 0.120-0.180 × 0.029-0.037 in all species		
size on arrival in liver, mm	about 0.160-0.200 × 0.023-0.040 in all species		

hydrate is probably the most important energy source for schistosomes. There is, in general, incomplete oxidation of energy-yielding substrates to fatty acids such as lactic, acetic, and proprionic acids via the Embden-Meyerhof pathway. It appears that oxidation is complete in the free-living stages (miracidia and cercariae) that do not live in energy-rich environments. The worms ingest red blood cells and possess a protease that releases tyrosine from globin and haemoglobin. The worms regurgitate a haematin-like pigment that is taken up by reticuloendothelial cells, mainly of the liver and spleen.

Eggs

The yellowish eggs are nonoperculate and have a spine, the size and position of which depends on the species. The spine may help the eggs to resist the blood flow when they are released and may also assist penetration of the wall of the venule. Histiolytic enzymes are released by the egg as it passes through the tissues to the lumen of the gut or bladder, during which time the contents mature into a fully developed miracidium. Many of the eggs fail to escape from the definitive host and are trapped in various tissues, especially the bowel and liver in the case of *S. mansoni* and *S. japonicum*, and the bladder and urogenital tract in the case of *S. haematobium*. Those eggs that do escape are voided in the excreta (faeces or urine) of the definitive host.

Hatching of the egg occurs in water and is generally believed to be stimulated by light at suitable temperatures (10–30°C) and low osmotic pressures. The miracidium becomes active and emerges through a slit in the egg shell caused by its own activity and by osmotic effects.

Miracidia

The miracidium is propelled through the water by cilia borne on 4 rows of epidermal plates. A primitive, apparently nonfuctional, bilobed or sac-like gut is present and 4 flame cells make up the excretory system. Muscles and germinal cells are present. A pair of prominent, unicellular " penetration " glands open at the base of the apical papilla. Penetration is initiated by the papilla, and the miracidium becomes attached to the body surface of the snail by a secretion from the gland cells. Lytic enzymes, as well as muscular effort, may be involved in penetration.

The miracidia of the schistosomes that infect man differ in size but are similar in their behaviour and morphology. They swim actively and are usually positively phototactic and negatively geotactic. The mechanism by which the miracidia of digenetic trematodes locate their molluscan hosts continues to be investigated, but is now thought to be dependent on chemicals produced by a potential snail host. Factors that influence the infection of snails by miracidia include: the age of the snails and miracidia; the temperature of exposure and the temperature of the habitat or microhabitat in which the snails live during the prepatent period; the duration of contact; water turbulence and flow; and ultraviolet light.

Mother sporocysts

The ciliated coat of the miracidium disappears and the muscles degenerate after penetration of the snail. Essentially, the parasite reforms into a nonmotile sac containing germinal cells that develop into daughter sporocysts. Slight, nonspecific, focal proliferative tissue reactions may occur around penetrating miracidia and may result in their death even when they penetrate the appropriate species of snail. The maturation of the daughter sporocyst is complete 10–15 days after the miracidium penetrates the snail.

Daughter sporocysts

These, unlike the mother sporocysts, are motile and pass to the digestive glands and ovotestis of the snail where they grow larger than the mother sporocyst and become entangled with the host tissues. The germinal cells develop into cercariae that break out of the daughter sporocyst and pass through the host tissues and blood sinuses of the snail to the edge of the mantle, whence they break out of the snail. Regeneration of the daughter sporocyst occurs and cercariae are produced over a long period. The daughter sporocysts provoke a tissue response intermediate between the focal and generalized types; cercariae may be trapped in the loose vascular connective tissue of the snail with an accompanying generalized proliferative reaction leading to the formation of granulomata.

The cycle within the snail intermediate host between the penetration of the miracidium larva and the production of mature cercariae lasts approximately 4–5 weeks for *S. mansoni*, 5–6 weeks for *S. haematobium*, and 7 or more weeks for *S. japonicum*.

Cercariae

The cercaria appears as a discrete head (the future schistosomulum) to which the elongated, bifurcated tail is attached to provide a locomotory organ.

Circular and spirally arranged longitudinal muscles work against the central tail core (hydroskeleton) to enable the tail to be moved vigorously during swimming. The tail also contains part of the excretory system. Small oral and ventral suckers are present on the head and can be used for creeping movements over solid objects and over the surface film of the water, as well as during the penetration of the vertebrate host. The cercaria is covered with a trilaminate tegument bearing minute hairs, spines, and a superficial layer of mucus. Internally there is a primitive, apparently nonfunctional gut, an excretory system of flame cells, a nervous system, and, predominantly, 6 or 7 pairs of unicellular glands. One pair, situated anteriorly, is emptied during escape from the snail. Three or four pairs of posterior, postacetabular (ventral sucker) glands secrete mucilage-forming particles on the surface of the oral sucker. These facilitate attachment and, at the time of penetration, may direct and prevent the loss of secreted enzymes. The external layer of superficial mucus is probably derived from these glands. The preacetabular glands empty during penetration of the vertebrate host, usually when the oral end has reached the keratogenous zone below the horny layer of the skin. The secretions disrupt physicochemically and possibly enzymatically the acellular cement and ground substances of the epidermis to facilitate penetration. The tail is shed during penetration.

Infected *Biomphalaria glabrata* may live for 2 months or more, but the normal duration of infection in other species is 1 month or less. Infection is normally terminated by the death of the snail, but some *Biomphalaria* and *Bulinus* spp. become spontaneously cured.

Among equally susceptible snails probably the most important factor determining output of cercariae is the size of the host, with large snails shedding more cercariae than small ones. Daily output may vary from 1 or 2 to several thousand cercariae. The snail hosts of *S. japonicum* are much smaller than those of most other species and therefore shed very few cercariae. At temperatures between 10°C and 30°C and possibly higher, light is the principal stimulus causing the release of cercariae of both *S. haematobium* and *S. mansoni*; cercariae are shed in smaller numbers in darkness, but periodic peaks of output occur even in the absence of light because of an innate rhythm. Prolonged exposure to light is apparently necessary for the stimulation of shedding in *S. japonicum*.

The effects of light, gravity, agitation, and touch on cercariae apparently increase the probability of infection of a definitive host, but it is the nature of the habitat and the behaviour of the host that chiefly influence the relative importance of these stimuli. Laboratory studies have shown that the greatest number of cercariae are infective to the vertebrate host at temperatures normally encountered in the field. The survival of *S. mansoni* cercariae decreases with age but is apparently independent of maintenance temperature between 12°C and 27°C. At 20°C–24°C cercariae of most species probably exhaust their glycogen reserves within 8–12 h. Their activity is rapidly impaired by exposure to ultraviolet light.

Schistosomula

The following criteria may be used to distinguish between a cercaria that has shed its tail and a schistosomulum. A schistosomulum has shed the cercarial glycocalyx, has empty gland cells, is killed by fresh water, and does not form pericercarial seroenvelopes in antiserum. It also soon assumes an elongated, wormlike shape.

The penetration process is quite rapid. Many cercariae penetrate the stratum corneum within a few minutes, and at the same time the larva changes in appearance to become a schistosomulum. After penetration the trilaminate tegument is replaced within a few hours by the multilaminate tegument of the adult.

The passage through the subcutaneous tissues is usually effected within 48 h. Peripheral lymphatic or venous vessels are then penetrated, from which transportation to the right heart and the lungs is accomplished. In mice, schistosomula can be found in the lungs after 4 days and they attain their highest concentration in this site in 5–7 days. Only a small proportion feed on red blood cells in the lungs and no growth takes place there. After 8 days a few are found in the portal vessels of the liver where active mitosis and growth takes place. Paired worms may be found in the mesenteric and portal veins after about 26 days. When sexual maturity is achieved growth slows considerably but still continues.

There is evidence to suggest that migration from the lungs to the portal system may not necessarily be via the circulation, but that some schistosomula pass directly through the diaphragm to the liver where they enter blood vessels of the portal system. Most worms leave the liver when they are sexually mature and have mated and, depending upon the species,

migrate to the mesenteric veins or to the veins of the vesical plexus where egg laying begins. The period between successful cercarial penetration and the appearance of eggs in the urine or stools of the definitive host may be 30–40 days, but is often considerably longer.

There is evidence to suggest that the presence of males is essential for the full maturation of the females, and there have been reports of the females of one species being aided by males of another species, both in maturation and transport from the liver to the mesenteric venules.

CLINICAL AND PATHOLOGICAL FINDINGS

A summary of common clinical and pathological findings is given in Table 2. A more complete description of the pathology is given in *General pathology*, p. 562.

HOST-PARASITE IMMUNOLOGICAL INTERACTIONS

The various stages of the parasite (penetrating cercaria, schistosomulum, adult worm, and egg) within the vertebrate host are all potentially antigenic. Possible antigens include: secretions, especially the histiolytic secretions of the penetrating cercaria and the escaping egg; digestive enzymes released during the expulsion of the digested blood from the gut of the adult; excretory products, especially those from adult worms; proteins released during turnover of the tegument; and the breakdown products of all stages that may die from any cause within the vertebrate.

A number of antigenic substances are therefore undoubtedly present in an infected vertebrate and these are known to produce humoral and cellular responses. These are discussed in detail in other sections, but the following general points may be made here:

(1) A response to any one stage of the parasite (or to its secretions and/or excretions) will not necessarily affect any other stage. For instance, the presence of eggs is not essential for the development of acquired resistance to schistosomula (see *Immunity in experimental animals*, p. 570).

(2) A specific response may not be lethal to the stage eliciting it.

(3) Even the smallest stages (egg, penetrating cercaria, and young schistosomulum) are multicellular and are not immediately susceptible to direct phagocytosis.

(4) A specific response to one stage of one species may not affect the same stage of another species or even that of a different strain of the same species.

GENERAL DISCUSSION

In a general discussion of the life cycle the following points are considered to be particularly relevant to immunological investigations:

(1) The variability of transmission patterns and therefore of the inoculum of infection may affect the immunological responses of the host. Infraspecific variations of the parasite and intraspecific variations of the definitive host may also influence the clinical, pathological, and immunological situation.

Table 2. Pathology of schistosomiasis

Stage	Parasitological	Clinical	Pathological
Invasion	penetration	cercarial skin reaction	papular dermatitis
	migration	fever and cough	inflammatory reactions in lungs and liver
Maturation	maturation, early oviposition and migration to definitive sites	acute febrile illness (often absent)	intense reactions, general and local, to products of eggs and/or young schistosomes
Established infection	intense oviposition: intense egg excretion	early chronic disease with haematuria or digestive symptoms	local egg reactions with granuloma formation but little fibrosis
Late infection	prolonged infection, often with decreased egg excretion	chronic disease, e.g., obstructive nephropathy, portal hypertension, cor pulmonale, etc.	progressive formation of fibrous tissue in various sites according to species

(2) In view of the poor state of knowledge regarding the route of migration of the schistosomulum both from the site of entry to the lung and from the lung to the site of maturity, further studies are considered necessary. Possible techniques are thoracic duct cannulation and labelling of cercariae or schistosomula.

(3) It must be stressed that, unlike bacteria or protozoa, the adult schistosomes do not multiply in the definitive host. In terms of host/parasite populations, the distribution of schistosomes within a population of vertebrate hosts is overdispersed. This implies that, although there will be a small proportion of heavily parasitized individuals, the majority of infections will consist of a few worm pairs. It should also be noted, however, that egg production involves an increase of parasite material within the host. The extent of this depends on the longevity of the adult, the proportion of the eggs retained in the tissue, and their rate of destruction. Despite numerous investigations involving tissue egg counts, there are few reliable estimates of the ratio of eggs retained to those excreted throughout the course of the infection.

(4) Relatively few studies are available on the metabolism and physiology of the schistosomulum. Such studies, especially those on the formation of the tegument and the synthesis of membrane antigens, are essential to the understanding of immune mechanisms acting on the surface of the invading parasites.

(5) It has been suggested that sperm released from the adult male into the blood stream will act as antigen. Virtually nothing is known about the reproductive processes in schistosomes. If sperm production could be maintained on a large scale *in vitro*, it might be possible to use this material for immunological studies.

RECOMMENDATIONS

(1) Studies on intraspecific variations of the parasite and on intraspecific variations of the definitive host should be continued and extended to an investigation of their effects on the clinical, pathological, and immunological presentation of the infection.

(2) Studies should be concentrated on defining the route of migration of the schistosomulum from the point of entry to the lung, and from the lung to the site of maturity.

(3) Studies should be made throughout the course of the infection on the egg production of the female worms and on the proportions of eggs excreted and retained in the tissues.

(4) Further studies should be made on the metabolism of the schistosomulum, with special regard to the synthesis of surface antigens.

(5) An attempt should be made to produce *in vitro* large numbers of sperm for use as a specific antigen.

(6) Further studies are required on the life-span of the adult worms. Similar studies are required for eggs, both those that are trapped in the tissues and those that successfully escape from the host.

(7) More information is required on the mechanism by which eggs pass through the tissues, and on the mechanisms by which eggs are destroyed in the tissues.

SCHISTOSOMAL ANTIGENS

There have been many investigations on the preparation of schistosomal antigens, but until now most have been hindered by inadequate methods of purification. Attention is now being paid to improved techniques for detecting, purifying, assaying, and determining the metabolic turnover of such antigens.

ADULT AND SCHISTOSOMULAR ANTIGENS

There is no accepted serologic or other *in vitro* test that correlates with protective immunity. Therefore, it is difficult to develop an experimental system for identifying and characterizing those schistosome antigens that are responsible for inducing protective immunity (the protective antigens). At best, only very poor protection can be induced by vaccination with dead parasite antigen; protective immunity develops only after the host has experienced a living infection, a fact that suggests that the stimulation of immunity is due to some metabolic process involving the release of protective antigens.

It is possible that some protective immunity is stimulated by the young migrating schistosomula, but the finding in monkeys and mice that immunity develops only slowly after a small initial infection is

good evidence that the adult worm is an important immunogenic source. Moreover, the surgical transfer of adult worms into normal monkeys has indicated that the living *adult* worm provides the major stumulus to protective immunity (see *Resistance to infection in experimental animals*, p. 570). On the other hand, studies have indicated that immune effector mechanisms are directed against *young* migrating schistosomula of the challenge infection. The recent development of the lung recovery assay (see *Resistance to infection in experimental animals*, p. 570) has provided clear evidence that a challenge infection is destroyed either in the lungs or at an earlier stage of migration.

It follows, therefore, that antigen produced by living adult worms stimulates an immunity that is directed against the young migrating schistosomula of a challenge infection. The adult worms themselves appear to be protected in some way from the immunity they provoke (see *Escape mechanisms*, p. 576).

How are the invading schistosomula killed? The surface membrane of the worm is the first line of defence against antibodies or immunocompetent cells, and surface damage could be expected to play a prominent part in the immune effector mechanism. Although systems that demonstrate antibody or cell-mediated damage to schistosomula *in vitro* do not as yet correlate with protective immunity, all *in vitro* systems that have been studied so far indicate immune damage to the schistosomulum surface. Further evidence that immunity damages the surface of worms has come from experiments demonstrating destruction of the surfaces of adult worms that had been transferred direct into the hepatic portal system of hyperimmune monkeys.

One approach to an investigation of the protective antigens of schistosomes, therefore, is to study the surface membrane proteins of the schistosomulum and the adult worm. An essential requirement for this study is the ability to isolate the surface membranes from the body in the parasite. In this context, experiments involving radiolabelling of isolated surface membranes or of antigens released into culture supernatants, followed by exposure to immune monkey serum and coprecipitation with anti-monkey IgG, have been carried out. Analysis of isolated membrane antigens from the schistosomulum and adult by polyacrylamide gel electrophoresis demonstrates the presence of many peptides of similar sizes in the two stages. Antigenic identity of these peptides is considered likely, based on the ability of adult worm membranes to absorb the antischistosomular activity of immune serum. However, it is suggested that formal confirmation of such identity should be obtained by techniques such as mixed agglutination.

The surface membrane of the adult worm has also been shown to undergo a process of turnover, involving release of proteins into the culture medium *in vitro* or loss of proteins from adult worms *in vivo*. These proteins form complexes with serum from monkeys that have developed immunity following infection, and such complexes can be precipitated by rabbit anti-monkey IgG. Substitution of normal monkey serum in these systems does not result in nonspecific coprecipitation of these membrane antigens. Sequential labelling with two isotopes indicate that the released proteins have a higher turnover rate than either the rest of the surface membrane or the soluble fraction of the worm. In contrast to the adult, schistosomula that are less than 19 days old release very little antigenic material.

It is suggested, therefore, that surface membrane antigens are released into the bloodstream by the adult worm, and that these released antigens stimulate an immune response that is directed against target antigens present on the surface of the young schistosomulum. It is also possible that the continuous high turnover rate of certain proteins may help the adult worm to evade the immune response of the host (see *Escape mechanisms*, p. 576).

It must be emphasized that both the schistosomulum and the adult have " naked " multilaminate external plasma membranes. Certain structural elements can be recognized and the surface can be damaged in immune hosts, but further research on the chemical nature of this tegument is necessary.

Recent advances have also been made in the identification and purification of other worm antigens not necessarily related to protective immunity. Analysis of worm extracts by two-dimensional immunoelectrophoresis reveals the presence of more than 60 separate antigens. Of these one component, a lipoprotein, is common to many helminths and may potentiate IgE responses to schistosomes in rats, as it does to some other worms. Another fraction is genus-specific for schistosomes, while a third is species-specific for *S. mansoni*. These last two antigens can be purified on immunoabsorbent columns; a similar affinity chromatographic technique has been used for the purification of schistosomal acetylcholinesterase. In addition, a globinase has been purified from adult worms.

Worm antigens can sometimes be demonstrated in

the serum or urine of infected experimental animals or human patients. A high-molecular-weight carbohydrate antigen can be found in the circulation of heavily-infected experimental animals, and the concentration of this antigen may be correlated with worm burden. Another antigen has recently been detected in the urine of heavily infected patients, and in some of these patients antibody reactive with this antigen is found in the serum. This antibody presumably circulates in the form of immune complexes.

EGG ANTIGENS

The pathogenesis of schistosomiasis mansoni appears to be largely due to granulomatous inflammation around schistosome eggs trapped in the host tissues (see *Egg granuloma*, p. 564). The embryo within the egg shell grows and differentiates for 6 days and then lives for another 2 weeks. During the latter period enzyme-containing secretions pass through ultramicroscopic pores in the shell and enable the eggs to move through the host tissues. These secretions can be seen in preparations stained with hematoxylin and eosin (the Hoeppli phenomenon), and have also been demonstrated by immunofluorescence with serum from infected animals. When living or lyophilized eggs are placed in serum from patients with schistosomiasis, circumoval precipitins appear. Histochemical studies have shown that the secretions contain proteins rich in sulfhydryl and tryptophan groups, diastase-resistant PAS-positive material, and lipids.

Previous experiments have shown that exposure to a heterogeneous, soluble egg antigen (SEA) preparation sensitizes experimental animals for enhanced granuloma formation in the lungs around intravenously injected eggs. This response can be induced by small amounts of the material without adjuvant. The antigenic activity is sensitive to trypsin and RNase treatment, but is resistant to DNase. Polyacrylamide gel electrophoresis reveals nine protein-staining bands. The thermal lability of the material remains uncertain.

More recently, radiolabelled SEA has been subjected to molecular sieve chromatography. Three major peaks (A, B, and C) can be resolved, and these have been further analysed by polyacrylamide gel electrophoresis. The ability of the fractions to induce delayed hypersensitivity and to react with antibody from infected mice in a precipitin test has been investigated. One band from peak B is associated with a strong precipitin reaction but does not induce delayed hypersensitivity. Another band from peak B does not react with precipitating antibodies, but induces marked delayed hypersensitivity. The latter band has very low radioactivity.

It has also been found that the activities responsible for the induction of delayed hypersensitivity bind to a Sepharose-concanavalin A affinity column and can be eluted with alphamethylmannoside. This suggests that the active moieties contain carbohydrate.

SEA has also recently been extracted from *S. haematobium* and *S. japonicum* eggs by homogenization and ultracentrifugation. *S. haematobium* SEA elicits moderate delayed footpad swelling in mice sensitized by intraperitoneal injection of *S. haematobium* eggs. In contrast, *S. japonicum* SEA elicits massive immediate footpad swelling but no delayed reactions in mice infected for 5–10 weeks. *S. japonicum* SEA, when injected intraperitoneally, does not sensitize mice to granuloma formation around *S. japonicum* eggs in the lungs. However, it does sensitize when injected subcutaneously, and it is particularly effective when combined with Freund's complete adjuvant.

SUMMARY

Immune protection is primarily stimulated by antigen associated with adult worms. There is considerable evidence that the adult and the schistosomulum share several antigenic properties; additional evidence for this could be obtained by tests such as mixed agglutination. Continuous turnover and release of adult surface antigen has been established; this process could be related to immunity as well as to adult worm survival and to the production of circulating antigen or immune complexes. Some progress has been made in the purification and detection of adult worm antigen, including metabolic products. Egg antigen is primarily, if not exclusively, involved in immunopathologic processes. Progress has been made in the characterization and purification of egg antigen.

RECOMMENDATIONS

Continued emphasis should be placed on the purification and isolation of schistosomal antigen, and on investigation of the responses it induces. It is also recommended that further studies should be carried out on :

(1) Analysis of the chemical properties of adult and schistosomular antigens, and identification of those antigens common to more than one stage and those specific for each stage.

(2) The release of worm antigen and the relationship of this process to the coating of the worm with host antigen.

(3) The isolation and chemical identification of the adult antigen that stimulates and potentiates IgE production.

(4) Further purification and chemical characterization of egg antigen mediating (a) antibody formation and (b) cell-mediated reactivity.

(5) An attempt to understand why egg antigen induces delayed hypersensitivity without requiring adjuvant.

PATHOLOGY AND IMMUNOPATHOLOGY

GENERAL PATHOLOGY

The pathology of schistosomiasis is essentially a series of chronic inflammatory lesions produced in and around blood vessels by eggs or their products, and sometimes by dead adult worms. The severity of clinical manifestations seems to be directly related to worm burden, since the focal vascular lesions can easily be repaired or compensated for unless they are disseminated and recurrent. In schistosomiasis mansoni, disseminated obstructive lesions of the portal radicles in the liver usually result in a syndrome of portal hypertension, with splenomegaly and the development of a collateral circulation. In some of these cases, pulmonary hypertension with cor pulmonale may ensue, following massive embolization of eggs and worms to the pulmonary vasculature. Renal and splenic changes, such as glomerulonephritis and follicular lymphoma, may also appear in patients with hepatosplenic schistosomiasis. Although these changes are not directly related to worms or eggs, they may depend on the schistosome infection.

All these major consequences of schistosome infection in man occur in about 4–12% of the infected population living in endemic areas. The large majority of infected people are either asymptomatic or show a mild nonspecific symptomatology, although they may be continuously exposed to the same environment as those with severe forms of the disease. Also, visitors to these endemic areas may have an acute febrile illness when first infected by the schistosomes.

In schistosomiasis japonicum the same organ systems are involved as are involved in *S. mansoni* infections. There are differences, however, owing to the different biology of the parasite. First, the worms produce 10 times as many eggs as *S. mansoni*. They are produced in large aggregates and have a tendency to calcify. Careful description of the pathology suggests a greater degree of exudative granulomatous lesions with more infiltration of neutrophils. In advanced cases a clay-pipestem fibrous-like lesion is seen. Another unusual aspect of *S. japonicum* is that the worm pairs tend to remain in one place and produce large masses of eggs. This may result in intestinal obstruction and, if ectopic organisms are found in the brain, large space-occupying lesions occur.

In schistosomiasis haematobium the urinary tract is largely involved, since the main habitat of the worms is the vesical plexuses. The worms produce approximately as many eggs as *S. mansoni*, but the eggs are released in aggregates and tend to calcify. The early granulomatous lesions are highly cellular, resulting in large space-occupying or polypoid lesions that may block the flow of urine and lead to the development of hydronephrosis. At later stages only relatively acellular fibrous lesions (" sandy patches ") occur. The calcification of the bladder that occurs in advanced cases is due to masses of calcified eggs. Evidence has been produced to show that in some areas cancer of the bladder occurs with greater frequency in patients with schistosomiasis haematobium. Involvement of the rectum and genital system has also been described. Eggs are often found in the liver and lungs, but significant pulmonary or liver disease is rare. When the central nervous system is involved lesions tend to occur in the spinal cord.

The spectrum of pathology of schistosomiasis may depend on many factors, one of the most important of which is the immunological response of the host.

Reactions to the schistosome eggs

The egg initially deposited by the female worm in the host tissue elicits no reaction until the miracidium matures (day 6) and the egg begins to produce a histiolytic secretion. Since the miracidium can

remain alive in host tissues for about 12 days, a complete focal granulomatous reaction may form while the miracidium is still viable and actively motile in fresh preparations. The material secreted by the miracidium is antigenic, and the binding of host antibodies to it can be demonstrated both *in vitro* and in cryostat sections treated with fluorescent conjugates.

In patients with acute schistosomiasis, and in animals with infections of 5 to 8 weeks' duration, the reaction around a viable egg is destructive. There is a periovular area of necrosis, with or without deposition of a hyaline eosinophilic band (Hoeppli phenomenon), surrounded by a predominantly exudative cellular reaction with many eosinophils. As the infection progresses and new viable eggs appear, the reaction around them gradually becomes smaller, more discrete, and purely proliferative, while the central necrosis and the eosinophilic periovular material tend to disappear. The most severe egg lesions occur during early infection, so that an initial massive infection produces severe clinical symptoms.

Rarely, an exaggerated fibroblastic reaction to focally-accumulated eggs may give rise to a pseudoneoplastic lesion. Patients with such lesions may even be operated on following a false diagnosis of malignancy, especially if they have obstructive intestinal masses. The cause of such a pseudoneoplastic formation seems purely local, since the host reaction to the schistosome eggs in other areas may be focal and limited.

Reactions to the worms

The adult worms ingest host blood that is later altered and regurgitated and can be seen as a PAS-negative, iron-free pigment in reticuloendothelial cells. Shed plasma membrane components and secretory and excretory substances produced by the worms may also be antigenic.

In tissue sections, live schistosome worms usually appear inside blood vessels unaccompanied by any local reaction. When the worm dies and disintegrates it may give rise to vascular thrombosis and inflammation. The reaction pattern evoked by dead worms is similar to that induced by eggs, namely a large, destructive-exudative, eosinophilic reaction appearing in early infection and a small, well-delimited, proliferative granuloma forming in late infection.

Chronic hepatitis

Chronic portal inflammation is a conspicuous and frequent occurrence in schistosomiasis. It is localized to the portal spaces and the fibrous septa derived from them, but the inflammatory cells may sometimes invade the liver parenchyma, dissociating and destroying the liver cells at the limiting plate (" piecemeal " necrosis).

In the liver, in addition to the egg granulomas, there is a diffuse cellular infiltration that in early infection is composed mainly of eosinophils. In acute schistosomiasis in man it has even been observed before oviposition has started. Later, especially when " pipe-stem " fibrosis develops, the cellular infiltration becomes patchy rather than diffuse, and mononuclear with lymphocytes and plasma cells predominating; there is a tendency towards perivascular localization and no correlation with the presence of worms and eggs. Sometimes, when portal inflammation is marked, with " piece-meal " necrosis and ductular cell proliferation, the reaction around the eggs may be scanty or absent.

Chronic hepatitis is especially prominent in cases of decompensated hepatosplenic schistosomiasis. In a comparative pathological study of the livers of subjects with compensated and decompensated hepatosplenic schistosomiasis it was noted that septal fibrosis, active periportal inflammation, and bile duct proliferation were significantly more marked in cases of decompensated than in cases of compensated hepatosplenic schistosomiasis. These changes are related neither to the number of eggs present in the liver nor to the presence of nodular regeneration or cirrhosis. Therefore, histological signs of disease progression seem unrelated to the intensity of infection at the time of death. Recently, it has been demonstrated in some areas that hepatitis B antigen (HB Ag) is found more frequently in patients with hepatosplenic schistosomiasis (4%) than in normal controls (0.8%).

Splenic changes

A series of changes occurs in lymphoreticular tissue, especially during schistosome infection. In recent infection there is proliferation of reticuloendothelial cells in the red pulp and in germinative centres of the lymphoid follicles. This is soon followed by multifocal proliferation of basophilic cells, many of them showing differentiation towards a plasmacytic line. These changes coincide with the elevation of the immunoglobulin concentration in the serum. Spleens kept outside the portal circulation, marsupialized under the abdominal skin, show the same pattern of changes. Later, the venous sinuses are distended owing to congestive changes produced by portal

hypertension, and the splenic cords become dense and thickened. In schistosomal splenomegaly it is usually assumed, therefore, that two factors are pathogenetically important: cellular proliferation and passive congestion.

In Brazil, in 1% of the spleens removed by splenectomy in proven cases of hepatosplenic schistosomiasis, a peculiar form of lymphoma has been reported, namely giant follicle lymphoma or nodular lymphoma, which is apparently primary and is limited to the spleen. The relationship of hepatosplenic schistosomiasis to this particular type of splenic lymphoma remains to be elucidated.

Renal changes

Nephropathies are quite frequent in patients with hepatosplenic schistosomiasis mansoni, glomerulonephritis being a common feature. In autopsy material chronic glomerulonephritis has been demonstrated in 12 out of 100 cases with hepatosplenic schistosomiasis, while its occurrence in cases of mild intestinal schistosomiasis was 5.6%. A systematic examination of kidneys from 100 autopsies performed in subjects with hepatosplenic schistosomiasis has disclosed a spectrum of changes which include mesangial thickening, mesangial cell proliferation, focal sclerosis, and several types of glomerulonephritis. The ultrastructural changes and immunofluorescence findings in such lesions are described in *Interactions of complement* (p. 566).

EGG GRANULOMA

The granuloma has been defined as a focal, chronic, inflammatory reaction in response to tissue injury by a poorly soluble substance, characterized by the accumulation and proliferation of reticuloendothelial cells. Studies over the past 10 years have provided much information on the etiology of this type of lesion in schistosomiasis. Most of these observations have been made with *S. mansoni*, but investigations of the *S. haematobium* and *S. japonicum* granulomas have also been carried out. It should be noted that granuloma formation around schistosome eggs tends to be similar in man and in most infected hosts in which it has been studied.

Eggs

In schistosomiasis the nidi around which the granulomas develop are schistosome eggs, trapped in the host tissues, that secrete biologically active materials. Circumoval precipitins form around eggs placed in serum from patients with schistosomiasis. Fluorescent antibody studies using immune serum from infected animals have revealed antigenic material surrounding the eggs, on the inner surface of the egg shell, and in the cephalic glands of the miracidium.

Etiology of the granuloma

The principal method used in the elucidation of the etiology of the schistosome egg granuloma is based on the isolation of eggs from the tissues of infected mice, followed by intravenous injection of these eggs into the pulmonary microvasculature of other mice. Eggs injected into animals previously sensitized by an intraperitoneal injection of eggs elicit a markedly accelerated and augmented inflammatory response. This reaction is highly specific and can be transferred from *S. mansoni*-infected mice by lymph node or spleen cells but not by serum. The high degree of specificity of this reaction has been confirmed by studies involving the different stages of the *S. mansoni* life cycle (eggs, cercariae, schistosomula, and adult schistosomes) and the three different species of schistosome egg.

Further investigations on the etiology of the granuloma have been greatly facilitated by the isolation of SEA by homogenization in tissue grinders and subsequent ultracentrifugation. Minute amounts of the supernatant, when injected intraperitoneally, can sensitize animals to granuloma formation around schistosome eggs. SEA has also been used to elicit delayed footpad swelling in mice and other animals. Granuloma formation can be elicited by injection of SEA, adsorbed onto bentonite particles, into the lungs of sensitized animals. Furthermore, SEA has been used as a test antigen for several different correlates of delayed hypersensitivity *in vitro*, including lymphocyte transformation, macrophage migration inhibition, and eosinophil stimulation. Active antigenic material has also been obtained by hatching the eggs in fresh water and by maintaining them in tissue culture fluid for long periods. It has been demonstrated that eggs totally depleted of SEA by prolonged maintenance in tissue culture lose their ability to induce granuloma formation.

Correlations

The *S. mansoni* egg granuloma has been correlated with other forms of delayed hypersensitivity both *in vivo* and *in vitro*. In the mouse a direct correlation between the onset of granuloma formation and delayed footpad swelling has been demonstrated.

Spleen or lymph-node cells obtained from infected mice incorporate thymidine or secrete MIF after exposure to SEA. In addition, intact granulomas isolated from the livers of infected mice and maintained *in vitro* secrete macrophage migration inhibitory factor and eosinophil stimulation promoter after exposure to SEA.

The onset and interrelation of four different parameters of delayed hypersensitivity after exposure to eggs has been studied in guinea-pigs. Granuloma formation, delayed skin reactions, and ^{3}H-thymidine incorporation into lymph-node cell suspension all begin in parallel 4 days after exposure; macrophage migration inhibition precedes the others by 1 day. During the first 10 days of immunization, during which delayed hypersensitivity develops fully, γ_1 and γ_2 antibodies are not detectable by passive cutaneous anaphylaxis and passive haemagglutination, respectively.

Suppression of granuloma formation

The effects on granuloma formation around eggs of many known and potential systems of immunosuppression have been tested. In every case those measures or conditions that have a greater tendency to suppress cell-mediated reactions (antilymphocyte serum, neonatal thymectomy, and Hodgkin's disease) inhibit granuloma formation, whereas those that tend to suppress reactions mediated by humoral antibody (irradiation and Friend virus leukemia) have no effect. Recent studies have shown that cholera toxin and niridazole, which strongly suppress cellular hypersensitivity but have little or no effect on antibody formation, markedly depress granuloma formation. Studies in chickens have shown that neonatal thymectomy strongly suppresses granuloma formation but that total hormonal bursectomy has no effect.

Specific immunosuppression in schistosomiasis has been reported in the offspring of heavily infected mice and in neonatal mice injected with soluble egg antigens. This tolerance-like phenomenon appears to be easily broken. Endogenous desensitization, a gradual waning of granulomatous hypersensitivity, has also been reported in chronically infected mice. Two possible mechanisms for this reaction are: immunological blockade induced by antibody or antigen-antibody complexes; or the selection of clones of cells that are so sensitive to antigen that they cease to be responsive (see *Escape mechanisms*, p. 576).

The immunological responses against SEA over a long period (1 year) of *S. mansoni* infection in the mouse change with time, and the opportunities for immunological interactions are numerous. During progressive disease the size and cellularity of newly formed granulomas diminish. Lymphoid cells or isolated granulomas from chronically-infected animals lose their ability to synthesize the lymphokines ESP (eosinophil stimulation promoter) and MIF. Likewise, a circulating, heat-labile reaginic antibody to SEA is no longer present. At the same time a heat-stable reaginic antibody to SEA appears in the serum and persists throughout the remainder of the infection. Positive lymphocyte blastogenic reactivity, peripheral blood eosinophilia, and elevated passive haemagglutinating antibody levels remain relatively stable throughout chronic infection.

If mice are depleted of their T lymphocyte population and are then infected with *S. mansoni* there are no unusual events until shortly after egg production. They continue to produce a thymus-independent anti-SEA antibody response, but no cell-mediated immunity or reaginic antibody is demonstrable. Such animals fail to produce the normal egg granuloma; instead, the eggs provide the nidi for multiple liquefactive necrotic lesions in the liver. The animals, which subsequently develop bacteraemia following such lesions in the gut, die sooner than intact animals that ultimately succumb to vascular obstruction attributable to granuloma formation. The mechanism of the necrotic lesions is incompletely understood and requires further investigation. Such lesions have not been observed after suppression of granuloma formation by antilymphocyte serum or by cholera toxin.

Granulomas in S. haematobium infections

Studies of the granuloma induced by injection of isolated *S. haematobium* eggs into the pulmonary microvasculature of mice have revealed that a relatively specific sensitization occurs. Passive transfer studies have not yet been performed.

Granulomas in S. japonicum infections

The granulomatous reaction to *S. japonicum* eggs, in contrast, does not have many of the characteristics of the *S. mansoni* or *S. haematobium* egg granulomas. Aggregates of *S. japonicum* eggs induce necrosis accompanied by large numbers of polymorphonuclear leucocytes. Plasma cells frequently occur in both the granulomas and the periportal infiltrates. Attempts to study the *S. japonicum* egg granuloma by the injection technique have previously failed

because the inflammatory reaction around the eggs is minimal both after primary and after secondary injections of eggs. Even when eggs are injected into the lungs of infected mice in which there are large granulomas in the liver, they elicit only minimal granuloma formation. Granulomatous hypersensitivity has finally been induced by the subcutaneous injection of both eggs and soluble egg antigens, and is particularly intense when these materials are injected in Freund's complete adjuvant.

IMMUNE COMPLEXES

The existence of soluble antigens (isolated from adult worms or eggs) and the demonstration of corresponding antibodies in subjects with schistosomiasis suggest the formation of immune complexes. The reaction of antibody with parasite antigen may occur: (a) at the site of localization of the parasite or its products; (b) in circulating blood in the case of soluble antigens; and (c) in extravascular spaces.

Immune complexes may be formed and persist in antibody excess as well as in antigen excess. Such complexes may cause vascular damage or lesions in tissues such as that of the kidney, but may also interfere with other mechanisms of host resistance.

Soluble immune complexes

Although sensitive techniques for the demonstration of circulating soluble immune complexes have been developed and used in other diseases, their application to schistosomiasis has been very recent. These include both techniques for detecting immune complexes regardless of antigen specificity, such as the $C1_q$ binding test and methods based on the existence of specific receptors for components of immune complexes on various cell lines, and techniques for detecting specific antigens bound in immune complexes (radioimmunoassay with labelled antigen or antibody).

Preliminary studies of schistosomiasis serum have revealed an increased binding of $C1_q$-I^{125} in some samples.

Interactions of complement

The titration of complement components has been carried out on limited numbers of serum samples from patients infected with *S. mansoni* and *S. haematobium*. The results have shown significantly decreased levels of some components and an increased concentration of the C_{3d} fragment of C_3 in most of the samples. The presence of immunoconglutinins was also detected in most of the samples. These data suggest an increased catabolism of complement components and the interaction of complement with immune complexes.

Immune complexes localized in tissues

The best evidence for localization of immune complexes in tissues has been established in the case of renal lesions. Early glomerular lesions in patients infected with *S. mansoni* (even those without clinically demonstrable renal damage) have been demonstrated by light, electron, and fluorescence microscopy. Immunofluorescence studies of renal biopsies have revealed the presence of immunoglobulins and complement; in these investigations, however, no antigen was demonstrated and no attempts were made to elute the antibody or estimate its specificity. Electron microscopy has also shown the presence of electron-dense material in the glomerular basement membrane; this is compatible with findings from immunofluorescence studies.

Glomerular lesions in experimental infections of *S. mansoni* have also been examined. In chimpanzees many kidneys were normal, and only some showed minor focal glomerular changes. Deposits of immunoglobulins in subepithelial and/or subendothelial areas of the glomerular basement membrane in *Cebus apella* monkeys were found 11–19 months after infection with 500 cercariae and repeated subsequent challenges. Noninfected controls did not show any lesions. Other workers, on the other hand, failed to find any significant deposits in kidney sections of baboons infected with 1 000–3 000 cercariae. Care should be taken in evaluating glomerular lesions in these animals since spontaneous lesions occur that increase progressively with age. Abnormalities range from focal thickenings of the basement membrane to more advanced lesions, progressing in older animals to chronic glomerulonephritis. Recently, proteinuria and histological changes have been demonstrated in kidneys of Swiss mice 2–3 months after infection with 80–100 cercariae. Granular deposits of immunoglobulins and the third component of complement were demonstrable in the glomerular walls in about two thirds of the animals, while the presence of *S. mansoni* antigens could be demonstrated by immunofluorescence in glomerular lesions in 36% of infected mice.

All these studies suggest the existence of lesions associated with immune complexes in *S. mansoni* infections. However, the nature of the antigen(s) involved is still poorly understood. The mouse ex-

periments indicate the possible role of "soluble fractions" of adult worms, but the participation of egg or other antigens cannot be excluded. Moreover, there is a possibility that DNA may be involved: precipitating antibodies to heat-denatured calf-thymus DNA and *Bacillus subtilis* DNA can be found in the serum of hamsters and patients infected with *S. mansoni*. The precipitin lines show reactions of identity with *S. mansoni* DNA extracts. Antibodies to single-stranded DNA in the serum of patients with schistosomiasis have also been found. The clinical significance of antibodies to single-stranded DNA is not yet known.

The most common renal lesions in *S. haematobium* infections, both in man and in the baboon, are hydronephrosis and pyelonephritis. Recent studies suggest the association of an immune complex type of nephropathy with *S. haematobium*. The evaluation of the involvement of *S. haematobium* antigen and of the epidemiological importance of these renal lesions will require further investigation.

Severe glomerular changes, associated with faint deposits of C_3 in mesangial areas, have been described in chimpanzees infected with *S. japonicum*, while milder changes occur in rabbits. The significance of immune complex deposition in other tissues is still unknown, but such a mechanism may be involved in the formation of granulomas, especially in *S. japonicum* infections.

EOSINOPHILS

Evidence is accumulating for the association of various immune responses with eosinophils. The frequent association of blood and tissue eosinophils with atopic allergic reactions has been cited as circumstantial evidence that eosinophils play a role in IgE-mediated immune phenomena. The almost universally observed combination of high titres of reaginic antibodies and eosinophils in most helminth infections adds to this speculation (see *Relationship between eosinophilia and reaginic antibodies*, p. 568). Specifically sensitized thymus-dependent (T) lymphocytes have been implicated in the direct induction of eosinophilia, and in the production of factors generated by interaction of antigen and specific lymphocytes. One such factor in turn reacts with homologous antigen-antibody complexes and becomes chemotactic for eosinophils. Another activity that is synthesized by lymphoid cells on specific stimulation has been called eosinophil stimulation promoter (ESP) and is classed as a lymphokine. Culture supernatant fluids that contain this activity also stimulate granulocytopoeisis. Moreover, antigen-antibody complexes are chemotactic for eosinophils, primarily by generating the split product of complement (5a). Eosinophils preferentially phagocytose IgE-containing immune complexes, and anaphylactic reactions result in the release of an agent, chemotactic for eosinophils, called ECF-A.

Presence of eosinophils during schistosome infection

Striking bone marrow and peripheral blood eosinophilia occurs during primary active murine infection with *S. mansoni*. Furthermore, it is possible in normal, uninfected mice to induce an eosinophilic episode by the injection of either schistosomal eggs or SEA. Peripheral blood eosinophilia is clinically prominent during human schistosomiasis. Many eosinophils are often found in the perioval granulomas observed in the tissues. Eosinophils are also found in the lesions in the lungs around migration schistosomula, and may possibly be involved in their destruction (see *Evidence from studies in vitro*, p. 574).

Relationships between cell-mediated immunity and eosinophils in schistosomiasis

Following findings that indicate that some situations involving immune eosinophils depend on sensitized T lymphocytes and cell-mediated immune capabilities, it has recently been shown that some episodes of peripheral blood eosinophilia during murine schistosomiasis mansoni require a functioning T lymphocyte population. The ability of lymphoid cells from *S. mansoni*-infected mice to produce the lymphokine ESP when exposed to SEA has also been described. This ability is transient in chronic infections, lasting only from about 8 to 14 weeks after infection. It is interesting to note that this corresponds to the most active period of granuloma formation. Culture supernatant fluids that contain ESP activity also contain a granulocyte-active, colony-stimulating activity (CSA). Control supernatant fluids do not contain either ESP or CSA activity. Various findings suggest that ESP is a product of stimulated T lymphocytes.

Eosinophils and egg granulomas in vitro

The role of the eosinophil is still unknown. Eosinophils are capable of phagocytic activity, but are usually quantitatively less efficient than neutrophils. Subsequent intracellular killing of ingested microorganisms also appears to be less efficient in eosino-

phils than in neutrophils. Another proposed function of eosinophils concerns their possible participation in inactivating some of the biologically active small molecules released in anaphylactic reactions. Recent information regarding the content of arylsulfatase (3.1.6.1) in eosinophils, and the action of this enzyme in " detoxifying " SRS-A, has revived interest in this field. In schistosomiasis tissue eosinophils are found within the hyperactive egg granulomas, often in close association with the eggs. Actual degranulation of eosinophils on the surfaces of eggs has been reported. Preliminary observations on the effects of eosinophils on eggs *in vitro* indicate that some destruction of the eggs occurs and that ingestion of egg shell material by eosinophils can be observed.

Eosinophils and egg granulomas in vivo

The production and characterization of a monospecific anti-eosinophil (AES) serum raised against mouse eosinophils provides a useful tool for investigation of the role of eosinophils *in vivo*. By selectively destroying eosinophils, AES permits the study of different inflammatory reactions in the absence of eosinophils and could possibly determine whether these cells are detrimental or beneficial.

Injection of *S. mansoni* eggs intravenously into mice induces a prominent eosinophilic response in the peripheral blood, in the bone marrow, and in the granulomas that form around the eggs in the lungs. AES administration arrests the peripheral eosinophilia following egg injection. In contrast, the eosinophil precursors in the bone marrow increase markedly. AES is therefore not only specific as regards the granulocyte cell type but is also stage-specific as regards the stage of maturity of the eosinophil. Electron microscope studies provide further support for the stage-specificity of AES. The granulomas that form around eggs in AES-treated animals are markedly smaller than those in control animals and are devoid of eosinophils. Consequently, the space-occupying effect of these granulomas is reduced, and this may be beneficial to the host. It remains to be seen, however, whether the long-term effect of eosinophil depletion, particularly in natural infection, will be beneficial or detrimental to the host.

Relationship between eosinophilia and reaginic antibodies

Many clinical and experimental studies have demonstrated the presence, often in high concentration, of IgE (reaginic-type) antibody during schistosome infection, frequently associated with marked eosinophilia. The specificity of these antibodies has most commonly been tested by the immediate skin test response against adult worm extracts, but reagins have also been induced by cercarial and egg antigenic preparations. The reaginic response in the mouse against SEA has recently been studied during the course of chronic (1-year) infection. There seem to be two types of skin-fixing reaginic antibody induced by SEA; one is heat-labile (56°C for 30 min), and the other is stable following such treatment. Both are destroyed by incubation for 4 h at 56°C. Titres of the heat-labile antibody reach a peak 10–12 weeks after infection and then decline, whereas heat-stable antibodies develop in 12–14 weeks and remain at a high titre throughout infection. The function of these reactivities is unknown; in fact, the roles of all reaginic antibodies during schistosomiasis are as yet completely conjectural. However, models currently under investigation may provide an answer to some of these questions. For example, it has recently been demonstrated (see *Evidence from studies in vitro*, p. 574) that IgE antibodies from infected rats play some role in the direct adherence of macrophages to schistosomula.

SUMMARY

Schistosomes cause vascular damage, inflammation, and fibrosis, especially by means of fertile eggs. The severity of disease is related to the worm burden. Approximately 4%–12% of patients in endemic areas show advanced disease, which may be represented by portal fibrosis of the liver and portal hypertension, pulmonary vascular obstruction, and cor pulmonale in *S. mansoni* and *S. japonicum* infections, and by lower urinary tract obstruction in *S. haematobium* infection. Associated conditions such as glomerular disease and splenic lymphoma and bladder carcinoma (in *S. haematobium* infection) may be related to the schistosome infection. In the large majority of patients the pathology of schistosome infection is represented by a few scattered granulomas around eggs in several organs.

The main lesion in schistosomiasis is the granulomatous reaction that occurs around living eggs. In the case of *S. mansoni* and *S. haematobium* eggs, the reaction observed in experimental animals appears to have a large delayed hypersensitivity component. In the case of *S. japonicum*, immune complexes appear to contribute to the inflammatory reaction elicited by eggs. During the course of schistosome infection in

man, as in experimental animals, the size of the granulomatous reaction around the eggs diminishes.

In schistosomiasis, eosinophilia and infiltration of the granulomas with eosinophils are common, and the degree of eosinophilia and the size of the granulomas can be reduced by administration of an anti-eosinophil serum. Two types of skin-sensitizing antibody reactive with SEA can be demonstrated. T lymphocytes from animals infected with *S. mansoni*, when exposed to SEA, produce a factor that stimulates eosinophils.

Histological, immunofluorescence, and serological studies provide evidence for the occurrence of extravascular and circulating immune complexes during the course of schistosomiasis in man and in experimental animals. Immune complex formation may contribute to some of the pathological manifestations of the disease (renal complications and granuloma formation), and may also interfere with the mechanisms of resistance to the infection. Further investigations are needed to evaluate the involvement of such complexes in the clinical expression of the disease.

RECOMMENDATIONS

(1) Biochemical and immunochemical characterization of the material secreted by the schistosome egg should be undertaken.

(2) The immunological and other tissue reactions to egg antigens should be further defined, with special reference to differences between host and parasite species. In particular, the reactions in human patients should be examined in detail.

(3) Attention should be given to the diminution in the size of granulomas occurring during the course of the infection. The underlying mechanism should be analysed further, e.g., the role of antibody of particular classes or subclasses or of immune complexes in diminishing cell-mediated responses.

(4) The presence and pathogenic role of immune complexes in experimental animals and man should be studied further, with special emphasis on identifying and characterizing the antigens and antibodies involved. Studies should include:

(*a*) Detection and quantitation of soluble immune complexes in serum and other body fluids by sufficiently sensitive techniques; their possible interference with mechanisms of host resistance; and conditions under which they localize in different tissues.

(*b*) More detailed analysis of deposited immune complexes in tissues at the cellular and ultrastructural level and of the significance of such complexes in modifying various lesions, such as the egg granuloma.

(*c*) Analysis of the serologic consequences of immune complexes (changes in the levels of immunoconglutinins, complement, its components, and their breakdown products).

(*d*) Evaluation of findings *a–c* with respect to the pathogenesis and prognosis of the disease, and to the effects of therapy.

(5) The epidemiology and pathogenesis of renal lesions and their association with schistosome infections should be critically evaluated.

(6) Studies should be undertaken on the possible involvement of autoimmune processes. These could include cross-reactivity with DNA, release of liver antigens, and formation of antiglobulins.

(7) Attempts should be made to ascertain whether follicular lymphoma of the spleen is associated with schistosomiasis infection in countries other than Brazil.

(8) Studies should be carried out on the effect of chronic transfer of antibodies from infected normal animals into infected T-cell-depleted animals, with particular reference to liver, lung, and kidney immunopathology.

(9) With respect to eosinophils, further studies are needed on:

(*a*) Analysis of the biochemistry of eosinophil activity in relationship to schistosome eggs.

(*b*) Possible activation of eosinophils by the lymphokine ESP.

(*c*) The determination of whether the interaction of eosinophils and eggs has a specific component, and whether eosinophils have F_c receptors or associated antibody.

(*d*) The problem of whether the eosinophil is an essential cell in the destruction of the egg within the granuloma.

(10) Further studies on the involvement of IgE on immunopathological processes are required.

IMMUNITY IN EXPERIMENTAL ANIMALS

RESISTANCE TO INFECTION IN EXPERIMENTAL ANIMALS

The assay and development of immunity in different hosts

Three experimental animals have been used more extensively than others for studying immunity to schistosomes: the rhesus monkey, the mouse, and the rat. *S. mansoni* or *S. japonicum* will readily infect the rhesus monkey and the mouse, and mature worms develop about 5 weeks after infection. The adult worms remain alive and produce eggs for many months, although if the rhesus monkey is heavily infected a large proportion of its worm burden is eliminated soon after the 13th week of infection. In both hosts, however, there is a gradual development of immunity to reinfection. Sixteen weeks after a small initial infection of 100 cercariae, most rhesus monkeys will develop a solid immunity to a challenge with large numbers of cercariae, and before this time a partial resistance to challenge can be demonstrated. In the mouse immunity is generally never absolute, but by 60 days after an initial exposure partial resistance to a homologous challenge infection can be demonstrated. *S. bovis* has also been used for immunity studies on mice, and it has been shown that 9 weeks after an initial infection with 100 cercariae only 34% of a challenge infection survives.

Because antibody or cell-mediated activities have not so far been correlated with protection, resistance to reinfection can only be measured by following the fate of a challenge infection. This is generally achieved by recovering the adult worms of the challenge, by perfusion from the liver, several weeks after the challenge has been given. The major disadvantage of this technique is the long period required for the parasites of the challenge to mature.

A more rapid assay of immunity in small animals has recently been developed that is based on the recovery of the schistosomula of a challenge as they traverse the lungs. In mice and rats, schistosomula of *S. mansoni* can be recovered from the lungs from day 3 onwards, with peak numbers on the fourth or fifth day. About 20% of a challenge can be recovered from the lungs of normal mice and 10% from those of normal rats 5 days after exposure. In animals that have developed immunity there is a considerable drop in the number of schistosomula recovered from the lungs after challenge, indicating that in immune animals a large proportion of invading schistosomula are eliminated in the lungs or at an earlier point in their migration. One advantage of the lung recovery assay is that the results become known soon after the challenge has been given, and they are therefore applicable to the condition of the host at the time of challenge and not several weeks later. A second advantage of the technique is the ease with which the worms of a primary infection may be distinguished from those of a challenge infection. This distinction may also be achieved by prelabelling the cercariae in the snails. After challenge, during most stages of the primary infection, the results of the lung recovery assay are consistent with those obtained by liver perfusion.

Fig. 1 illustrates the development of acquired immunity in mice and rats following a primary infection, as measured by the lung recovery technique. The animals were challenged at regular intervals after an initial infection and the number of schistosomula recovered from the lungs on day 4 or 5 was compared with the number recovered from control animals. It can be seen that immunity to reinfection follows a different pattern in the two hosts.

In the rat, immunity can be demonstrated at week 3; it reaches a peak between week 6 and week 7 and then declines to zero. After a subsequent challenge resistance reappears very quickly.

In the mouse, immunity is less efficient and is slower to develop; a plateau is not reached until week 12 or later. The decline in immunity in the rat may be explained by the fact that most worms from this host are eliminated between weeks 4 and 8, and it is possible that a continual antigenic stimulus is needed to maintain immunity at a high enough level to enable a challenge infection to be destroyed at or before the lung stage. The temporary phase of low recovery of a challenge infection from the lungs of mice during the first 3 weeks of a primary infection reflects a delay in the migration of the parasite rather than protective immunity.

Concomitant immunity

An important feature of acquired immunity in

experimental hosts is that established adult worms from the initial infection persist long after resistance has developed against a challenge infection. In the rhesus monkey and the mouse, for example, a small infection with *S. mansoni* stimulates an immunity that prevents (or in the mouse partially prevents) further reinfection with cercariae. However, the adult worms from the original immunizing infection live on despite this immunity and viable eggs can be demonstrated in the faeces during and after the destruction of the challenge infection. A similar situation has been reported in baboons infected with *S. mansoni* or *S. haematobium*.

Clearly, whatever the nature of the immune response that prevents the challenge from maturing, in most cases it does not at the same time destroy established adult worms or prevent them from producing eggs. The existence of immunity in the presence of an active infection is considered to be an example of concomitant immunity.

Although the adult usually evades the immune responses of the host, damage to the adult may sometimes occur. After direct transfer of adults from normal to hyperimmunized rhesus monkeys, damage to the tegument occurs. In baboons immunized with *S. haematobium* egg-laying by adult worms is markedly reduced, but after transfer to normal hosts normal egg-laying is resumed. Spontaneous elimination of adult worms a few weeks after a primary infection occurs in rhesus monkeys and rats, and possibly in other species. Whether or not this process is immunological in nature remains to be determined.

Heterologous immunity

Acquired immunity is intraspecific. Immunization of rhesus monkeys or mice with cercariae of the zoophilic strain of *S. japonicum* induces immunity against a challenge with the human strain. Immunity can also develop across the species barrier. For example in rhesus monkeys *S. bovis*, *S. mattheei*, and *S. japonicum* will induce protection against *S. mansoni*, in the baboon *S. rodhaini* and *S. bovis* will immunize against *S. mansoni*, and in mice *S. bovis*, *S. mattheei*, and *S. rodhaini* will protect against *S. mansoni*.

Immunity will also develop after exposure to a schistosome of a different genus from that of the challenge infection. Thus *S. mansoni* protects against *Schistosomatium douthitti* in mice and *Ornithobilharzia turkestanicum* protects against *S. bovis*, *S. haematobium*, and *S. mansoni*.

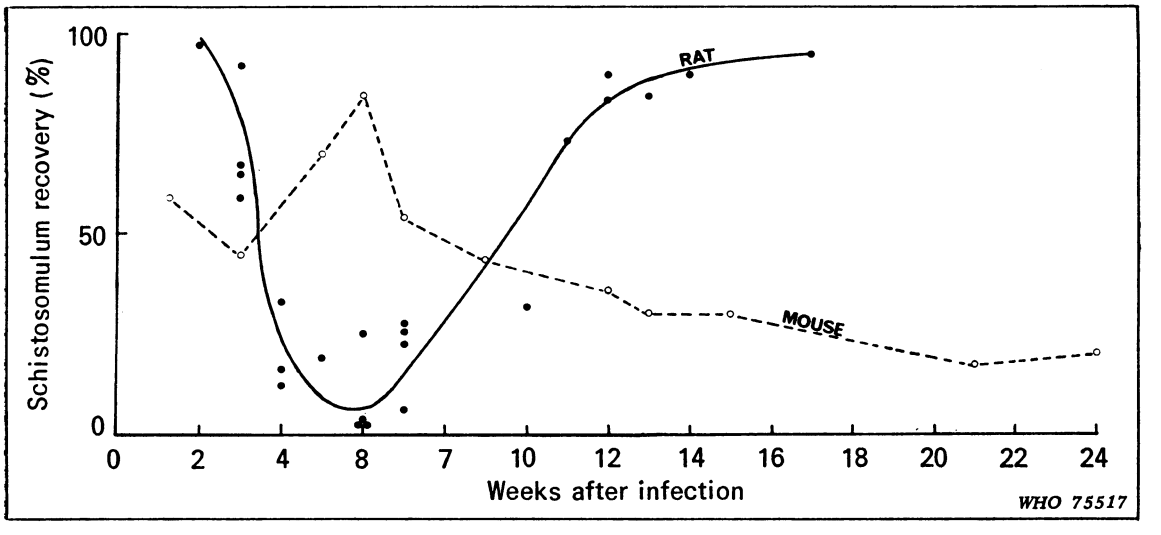

Fig. 1. The development of protective immunity to *S. mansoni* in rats and mice following a primary infection. Groups of animals were challenged with cercariae at intervals following a primary infection and immunity to the challenge was assayed by the lung recovery technique. The lower the recovery of schistosomula from the lungs the greater the degree of immunity. (Reproduced from Smithers, S. R. & Terry, R. J. *Advances in parasitology*, **14**: (1976) (in press) by kind permission of Academic Press.)

Some workers believe that the degree of cross-protection is correlated with the phylogenetic relationship of the schistosomes. Other workers, however, could demonstrate no protection in mice between *S. bovis* and *S. mattheei*, two closely related schistosomes, although *S. bovis* did protect against the more distantly related *S. mansoni*. This led to the belief that in some cases heterologous infections produce a more violent rejection reaction than does the homologous parasite. A heterologous system that produces a markedly better immunity than its homologous counterpart could be important in the development of living and dead vaccines, and further comparative studies are needed on the immunity induced by heterologous and homologous infections.

The stimulation of acquired immunity

The general failure to provoke immunity by injecting nonliving parasite homogenates has inhibited progress towards identifying the antigenic stimulus to immunity, and our understanding of the stimulation of immunity has necessarily been confined to the living parasite.

The schistosome egg. Although the egg is a strongly antigenic stage in the life cycle, there is convincing evidence that it plays no role in inducing a protective response. Unisexual infections of *S. mansoni* and *S. japonicum* do not lead to egg production; nevertheless, rhesus monkeys and mice become resistant to challenge following infections with one sex only. Moreover, the injection of viable eggs into the circulation fails to stimulate resistance to *S. mansoni* in mice. Similar results were obtained when 500 000 living eggs were injected directly into the mesenteric veins of rhesus monkeys.

The schistosomulum. The immunizing effect of irradiated cercariae has been studied for both *S. mansoni* and *S. japonicum*. There is general agreement that doses of radiation greater 2 000 R prevent the majority of worms from developing into mature egg-laying adults; the schistosomula die mainly in the liver 2-4 weeks after infection, although if the radiation dose is high (e.g., 48 000 R) the schistosomula are destroyed in the lungs or skin. Any immunity induced by exposure to irradiated cercariae is generally attributed to the presence of the schistosomular stage of the life cycle, although it is very difficult to exclude the effect of the few worms that may survive irradiation and remain alive as stunted adults. The best immunizing effects with irradiated cercariae have been obtained with *S. japonicum* in rhesus monkeys that had been inoculated 3 or 4 times with large numbers of cercariae irradiated at 1 700–3 000 R. The fact that a period of 6 months is necessary between the final immunizing inoculation and challenge in order to obtain the highest degree of immunity in this system suggests that the effect is more likely to be due to the presence of stunted adults than to migrating schistosomula. Partial immunity to *S. mansoni* can be induced in the rhesus monkey after repeated exposure to large numbers of irradiated cercariae, although one exposure to 25 000 cercariae irradiated at 5 000 R does not protect. Immunity to *S. mansoni* in mice is induced by similar means, although there is some disagreement as to the number of irradiated cercariae and the number of exposures necessary.

The fact that vast numbers of irradiated cercariae given in several spaced inoculations is necessary to induce some protection in rhesus monkeys, whereas a single exposure to as few as 100 or 200 normal cercariae stimulates an absolute immunity, suggests that, in this host/parasite system at least, something more than a short-lived schistosomulum is required in order to induce protection. These findings do not apply to all host/parasite systems; for example, irradiated cercariae are as immunogenic as normal cercariae in the rat.

The adult. The fact that immunity in monkeys and mice develops slowly after a small initial infection is good evidence that the adult worm is an important immunogenic source. Direct evidence of the role of the adult in stimulating immunity has been demonstrated by the transfer of *S. mansoni* adult worms from other hosts into the hepatic portal systems of normal monkeys, which induces an adult infection without prior exposure to cercariae or migrating schistosomula. Monkeys treated in this way with about 80 pairs of worms were almost completely resistant to challenge with cercariae 8–14 weeks later. Worms killed by snap-freezing immediately before transfer did not induce immunity, whereas worms cut transversely into two immediately before transfer did so. The anterior ends of these cut worms survived for several weeks but were unable to produce eggs. This result provides further evidence that immunity can be induced by stunted adult worms in the absence of egg production. It is not known whether the persistence of the adult worm is necessary for the maintenance of immunity.

The cercaria. The majority of experiments that have investigated and demonstrated acquired resistance

have been within the framework of long-term infection. However, although these methods may indicate how immunity develops during natural infection, they are not aimed at investigating other potential means of artificial immunization that may depend upon mechanisms (antigen-immune response systems) other than those operative during natural infection. It has recently been reported that multiple exposures to *S. mansoni* cercariae led to positive skin tests (early and late reactions) and lymphocyte blastogenesis against a cercarial antigenic preparation whereas a single infection did not. These responses developed prior to patency. Their relationship to resistance is not yet established, but the ability of suitable exposure to early stages to stimulate responsiveness to early-stage antigens may reflect a different set of responses than is stimulated late in infection, and such potential methods could be explored more fully.

POSTULATED MECHANISMS OF ACQUIRED RESISTANCE

Evidence from studies in vivo

Although it has been known for many years that a wide variety of experimental animals acquire resistance to schistosomiasis in response to previous infection with the parasite, it is only recently that we have begun to define the immunological basis of the phenomenon. Clearly, schistosomes are able to provoke a multitude of different humoral and cellular responses, and as targets *in vitro* they are susceptible to damage by several distinct immunological effector mechanisms. The difficulty exists in relating these immune responses induced by the parasite to the process whereby schistosome infections are eliminated in resistant animals. Therefore, the analysis of acquired resistance as it occurs *in vivo* has become a crucial field of investigation in the attempt to evolve a strategy for inducing protective immunity in man.

A recent emphasis in the work of several laboratories has been the study of acquired resistance in small animal hosts, namely, the rat and the mouse. The convenience of these rodent species as experimental models for the analysis of immunity to schistosomes cannot be stressed too strongly. By means of laboratory rats and mice, workers have been able to use experimental approaches such as adoptive transfer, depletion, and reconstitution which would be neither technically nor financially feasible in primates. It is to be hoped that the present research on rodent schistosomiasis will establish a framework of immunological concepts, the relevance of which will be directly testable in higher animals and in man.

Recent reports indicate that during the *early* stages of primary infection the acquired resistance to reinfection may involve a specific cell-mediated response. However, most of the current research is focused on an analysis of the mechanism functioning during the period after primary infection when acquired resistance is at its most effective level, i.e., 5–7 weeks in the rat, 12 weeks and later in the mouse.

At this stage, animals appear to reject challenge infections of *S. mansoni* by a mechanism with the following immunological components:

Antibody. Passive transfer experiments in the rat, mouse, and rhesus monkey indicate that the effector mechanism of acquired resistance involves a humoral component. Serum from rats infected for 5–7 weeks, or from mice infected for 12–16 weeks, transfers to normal recipients an average of 47% of the level of resistance present in the donors. The data are similar to those reported for the passive transfer of immunity against other helminth infections (*Fasciola hepatica, Nippostrongylus brasiliensis, Trichuris muris*) in that large volumes of serum must be injected and that a percentage of the serum pools collected appear to be biologically inactive. Although the factor in immune rat and mouse serum responsible for passive transfer has not been characterized completely, the activity is present in chromatographic fractions of serum rich in immunoglobulins of the IgG_1 subclass.

Complement. The complement system may also play a role in the effector mechanism of acquired resistance. Preliminary studies indicate that previously infected rats lose their ability to reject schistosome challenges when made deficient in complement by pretreatment with cobra venom factor.

Cells. Attempts to transfer immunity adoptively with lymphoid cells taken from animals at the height of their resistance have so far failed. Furthermore, resistance to reinfection in both the rat and the mouse is not blocked by treating animals with antilymphocyte serum at the time of challenge. However, in the rat the effector mechanism clearly requires the participation of a population of cells unsensitized at the time of challenge. This cellular component is sensitive to irradiation. Reconstitution of the effector function depleted by whole body gamma irradiation can be accomplished with cells from the bone marrow and lymphoid organs. Recent

work in which different doses of irradiation were used suggests that two different unsensitized cell populations may be involved. The passively acquired resistance of recipients of immune serum is also blocked by irradiation.

An important aspect of the effector mechanism described in these experiments is that it seems to operate against schistosomes during the first few days of their development in the host. Both the passive transfer and radiation depletion procedures are therefore significantly less effective when administered to animals after the first 24 h of the challenge infection. These findings may indicate that the surface of the developing schistosomulum is susceptible to immune attack for only a limited period, or alternatively that rejection occurs at a specific anatomical site (e.g., the skin or lungs) through which the parasites pass during the early stages of their migration in the host.

In addition to the humoral and cellular components for which there is now direct evidence, other factors may contribute to the effector mechanism of acquired resistance. The fate of schistosome challenges could conceivably be influenced by a variety of nonspecific effects produced in response to adult worms of the primary infection. These might include processes such as macrophage activation or eosinophilia.

In summary, the evidence now available suggests that, in some experimental animals, acquired resistance during its most efficient phase employs an effector mechanism involving the cooperation of a minimum of three components: antibody, complement, and unsensitized cells. The exact process whereby these interacting elements mediate the rejection of challenge infections remains unresolved. Two possibilities are:

(a) A direct cytotoxic effect against the surface of the schistosomulum mediated by an antibody-dependent cellular mechanism similar to those described *in vitro*.

(b) Damage or trapping by an inflammatory process generated by the interaction of antibody with parasite antigens released into local tissue sites.

Clearly, we need to learn more about the nature of the components comprising the effector mechanism and the schistosome antigens that serve as its target.

Evidence from studies in vitro

Although investigations *in vivo* provide the only certain means of identifying the mechanism of resistance to reinfection, experiments *in vitro* are important in that they offer a way of identifying possible effector mechanisms, the relevance of which may subsequently be tested by appropriate experiments *in vivo*. The techniques available are as follows:

Culture methods for schistosomula. Young schistosomes acquire a coating of host material soon after penetration, and this material may protect the worms from subsequent damage by the host's responses (see *Escape mechanisms*, p. 576). If so, acquired resistance to infection must be mediated by effector mechanisms that can act on the organism soon after it penetrates the skin. Most workers have therefore chosen the schistosomulum stage for experiments *in vitro*. Schistosomula can be obtained in large numbers by allowing cercariae to penetrate an isolated preparation of rat or mouse skin, and they may then be maintained for long periods in culture. During this time they grow and undergo partial maturation, and after subsequent transfer direct into the hepatic portal vein of suitable hosts they continue to develop into adult worms.

Schistosomula prepared *in vitro* are not necessarily identical to those found *in vivo*, and inadequate culture conditions may markedly accentuate the damage induced by any particular effector mechanism. Considerable caution is therefore necessary in the interpretation of effects seen *in vitro*; these may not occur, or may be repaired, *in vivo*.

Assessment of damage to schistosomula. Death of the organism is associated with loss of motility and changes in gross morphology in that the organism becomes opaque and granular. Moreover, dead organisms fail to exclude eosin and Evans blue, and show altered staining patterns with fluorescein esters. These differences in staining properties may assist in the enumeration of dead organisms, but microscopical techniques of this type are always liable to a certain degree of subjective error, particularly when host effector cells are present. In such cases the host cells may obscure possible changes in the parasite.

Schistosomula may be labelled with ^{51}Cr as sodium chromate in the same way as single-cell suspensions of mammalian or avian target cells. Living organisms retain their label well and control preparations lose only about 20% of the incorporated chromium over a 15-h incubation period. Damage to the organism is associated with further loss of isotope but under conditions of extreme damage, such as repeated freezing and thawing in

hypotonic solutions, only 50–60% of the incorporated isotope is released. This means that the assay is less sensitive for schistosomula than for mammalian cells, since the working range of isotope release is only 30–40%. On the other hand this assay is objective and extremely reproducible, and has the advantage that a large number of samples can be handled in one experiment.

Schistosomula prepared *in vitro* and treated with antibody and complement may be introduced intraperitoneally into normal mice. After 8 weeks the mice are killed and the worms recovered by peritoneal washing and perfusion of the hepatic portal system are counted. This technique is potentially very useful, since it provides a measure of the number of worms capable of reaching maturity after the test procedure *in vitro*. However, variability between replicates is high, and the recovery of worms from animals that have received control preparations of schistosomula is unacceptably low.

Mechanisms of damage to schistosomula in vitro. It has been shown that fresh serum samples from rhesus monkeys that have received 2–4 exposures to cercariae over a 2- to 3-year period are toxic for schistosomula *in vitro*. In the presence of 50% fresh serum the percentage of dead schistosomula rises from 10–40% after 1 day of culture to almost 100% after 4 days. In cultures containing control serum less than 10% of the organisms are dead after 4 days. The lethal activity is associated with an IgG fraction of Sephadex G200 and QAE Sephadex, and depends for its effect on the presence of heat-labile components, presumably complement, present in normal serum. Concentrations of fresh normal serum of 25–50% are required to restore completely the lethal activity of heat-inactivated immune serum.

Antibody levels in serum from hyperimmunized rhesus monkeys, expressed as the dilution required to kill 50% of the schistosomula over 4 days of culture, range from 1 : 8 to 1 : 64. Lethal antibody is first detectable 20 weeks after primary infection. Resistance to reinfection, however, can be demonstrated in rhesus monkeys by 16 weeks after primary infection. Serum from animals infected for 16 weeks shows no lethal activity, but has a growth-inhibitory effect that is independent of complement.

Schistosomula that have been incubated for 1–4 days in medium containing normal serum, or that are recovered from mice 1–4 days after cercarial infection, are protected against both the lethal and growth-inhibitory effects of immune serum. This implies that the worms acquire their protective coating of host material very soon after penetration of the skin (see *Escape mechanisms*, p. 576).

Similar complement-dependent lethal antibodies have been demonstrated in infected human patients and in infected rats, and it was hoped that such antibodies might prove to be responsible for the resistance to reinfection seen in experimental animals. Recent observations, however, strongly suggest that this is not the case, at any rate in rats. A membrane antigen has been isolated from adult schistosomes that absorbs the lethal antibody activity from the serum of infected rats and rhesus monkeys. This antigen, when administered to rats, elicits the formation of lethal antibody in titres comparable to those seen in infected animals; it fails, however, to induce a detectable resistance to infection, as assayed by the recovery of adult worms 28 days after a challenge exposure.

A second complement-dependent effect on target schistosomula has recently been described. Fresh serum from infected rats, in the presence of polymorphonuclear leucocytes isolated from the peripheral blood of normal animals, kills schistosomula (determined microscopically) over a period of 20–44 h. Death is associated with the attachment of polymorphonuclear leucocytes and the release of lysosomal enzymes onto the schistosomular surface, as judged by staining with nitro blue tetrazolium. When low effector cell concentrations are tested the death of organisms is frequently associated with discharge of enzymes from only a single polymorphonuclear leucocyte. At high cell concentrations an " all-or-none " effect is observed: schistosomula are either dead and completely ensheathed by cells, or alive and completely free from attached cells. The antibody activity is associated with an IgG fraction of DEAE, and is dependent on the presence of 33–50% of fresh normal serum. In the presence of complement alone, without added cells, this serum causes relatively little death of schistosomula over a 44-h period. However, the time course of development of activity in infected rats is comparable with that described for complement-dependent lethal antibody, and there is no clear evidence that the two activities are different. It is possible that the polymorphonuclear leucocytes simply accelerate the damage initiated by complement. Both activities remain high after the " self-cure " that occurs in rats 4–8 weeks after infection, while resistance to reinfection wanes.

A factor has recently been demonstrated in heat-

inactivated serum from infected human patients that induces the release of ^{51}Cr from labelled schistosomula in the presence of normal human peripheral blood leucocytes. Release of chromium occurs over a 15-h period at effector cell/target cell ratios as low as 100 : 1, and a significant effect can be detected with some sera at a concentration of 1 : 300. Recent observations have indicated that the antibody activity is associated with an IgG fraction of DEAE cellulose, that the effector cells are concentrated in the polymorphonuclear leucocyte-rich pellet after centrifugation over Ficoll-Hypaque, and that similar activities can be detected in serum from infected baboons and mice. The relationship between this activity and the complement-dependent effects described above has not yet been determined, and the exact nature of the effector cell is not yet clear. Preliminary evidence that the eosinophil may be the effector cell requires further investigation.

A further mechanism has been described, involving the cytotoxic effect of rat macrophages sensitized with cytophilic antibody. Peritoneal exudate cells from normal rats are preincubated with *heat-inactivated* serum from immune rats at 4°C for 4 h. These cells are washed, and can be shown to adhere to and damage schistosomula over a 20-h incubation period. Immune macrophages, normal macrophages alone, or normal macrophages preincubated with normal rat serum, fail to show the effect. The development of the antibody activity in rats parallels the development and decline of immunity, and the activity can be distinguished from lethal antibody by separation on QAE Sephadex. The possibility that the cytophilic factor may be an immune complex has yet to be investigated.

A second system involving rat macrophages has also been described. Macrophages are incubated at 37°C for 3–14 h with *noninactivated* immune serum. They are then mixed, without washing, with target schistosomula, to which they adhere. Damage to schistosomula usually observed in the presence of immune serum alone is greatly accelerated by the addition of these preincubated macrophages. The activity in serum is progressively abolished by heating at 56°C for 30–180 min, and is not restored by the addition of complement. The activity can be shown to be antigen-specific, and is absorbed out by IgE specific antiserum but not by antiserum specific for other classes of immunoglobulin, namely, IgG (1, 2a, 2b), IgM, and IgA. The activity can also be removed by passing serum through IgE-specific solid phase immunoabsorbent columns. Again, the possibility that IgE-antigen complexes may be involved remains to be determined. This is the first time that IgE antibodies have been implicated in macrophage adherence reactions.

There are no reports of cytotoxic effects mediated directly by lymphoid or other cells from infected animals.

Conclusions. It should be emphasized again that results derived from observations *in vitro* cannot be interpreted directly in terms of resistance to infection *in vivo*. This is particularly true of schistosomiasis. If the assumption that worms acquire a protective coating of host material very soon after cercarial penetration is correct, any host defence mechanism that is effective in mediating resistance to infection must be able to act on the schistosomulum immediately after penetration. Any generally detectable systemic response may therefore be irrelevant. Hence it is not sufficient simply to demonstrate a particular cytotoxic effect *in vitro*; it is also necessary to show that the same mechanism can act *in vivo*.

This has not yet been achieved, but the studies described above concerning the complement-dependent lethal antibody provide a good example of the sort of approach that may be used. *In vitro* techniques, by virtue of their ease of manipulation, provide a convenient way of identifying responses that *may* be related to resistance to infection, and of analysing such responses in considerably more detail than would be possible in a model system *in vivo*. Only the simpler mechanisms have so far been examined in this way, and it may become necessary to think in terms of more complicated cooperative processes, such as the production of specific antibody together with nonspecific enhancement of the function of a particular effector cell type such as the eosinophil.

In summary, techniques are now available for studying the effects of the host's responses on schistosomula *in vitro*. Several mechanisms for damaging schistosomula have been described, and although there is no evidence so far that any of these mechanisms are effective *in vivo*, the techniques provide a useful tool for investigating putative protective responses in some detail.

ESCAPE MECHANISMS

The ability of adult schistosomes to survive in the blood stream of their hosts for months or even years implies that the worms are able to escape the consequences of the host's immune response. More

than one mechanism may be operating to enable the worms to survive.

Endogenously synthesized host antigens

Shared antigens between schistosomes and their definitive and snail hosts have been demonstrated by gel diffusion techniques. It is believed by some workers that some host antigens are of parasite origin and have evolved under selection pressure to resemble antigens of the host; this would reduce the overall immunogenicity of the parasite and favour its survival. This hypothesis does not easily fit a parasite like the schistosome that has a wide host range. To explain this, it has been suggested that schistosomes may have the genetic capacity to synthesize antigens of several host types, and that the correct genes for the particular host being parasitized are induced by some stimulation from the host. An example of a host-like antigen that is synthesized by the parasite is a surface antigen that cross-reacts with mouse α_2-macroglobulin and that is present on worms from both mice and monkeys. Since primate α_2-macroglobulin does not cross-react with mouse α_2-macroglobulin, this antigen is obviously synthesized by the parasite in any host. Another parasite-synthesized host antigen has been demonstrated by incubating schistosomes from hamsters *in vitro* with labelled aminoacid precursors; under these conditions the worms synthesize a protein that cross-reacts with hamster liver protein.

Acquired host antigens

Host antigens have been studied in a system *in vivo* involving the transfer of worms from mice to monkeys immunized against mouse red blood cells or other mouse antigens. These transferred worms are killed by an antibody-mediated reaction directed against the surface of the parasite.

These experiments strongly suggest that antigens are acquired from the host and incorporated into the surface of the schistosome. These host antigens have been studied further by growing schistosomula in a culture system containing human serum and red blood cells. When such cultured schistosomula were transferred to monkeys immunized against human red blood cells the parasites were killed. These experiments suggested that blood group substances are host antigens. Further analysis of the nature of the host antigens has been made by using the mixed agglutination and fluorescent antibody techniques on schistosomula cultured in the presence of human blood of known specificity. Such techniques have shown that only the A, B, and H blood group substances, and possibly the Lewis antigen, are acquired by the worm; there was no evidence that other blood group substances, such as the rhesus factors and the K, Fy^a and S antigens, were acquired. It should be noted that the former substances exist both as glycolipids and glycoproteins, whereas the latter are all glycoproteins.

Further evidence that host antigens are acquired as glycolipids has been obtained by the use of non-secretor serum, which contains glycolipid but apparently no glycoprotein, that can act as a source of host antigen. Furthermore saliva, which is likely to contain only glycoprotein blood group substances, does not act as a source of host antigen. On the other hand, an aqueous butanol extract of A-type erythrocyte ghosts, which according to recent evidence contain only megalolipid molecules with A activity, transfers blood group A to the worm surface.

The expression of such specific antigens as the human A, B, and H blood group substances makes it unlikely that the parasite synthesizes these particular host antigens. Evidence of the transfer of material from the erythrocytes to the schistosomula in culture has come from experiments using red blood cells in which the terminal galactose or galactosamine sugars are radiolabelled. Culture of schistosomula with the labelled red blood cells has demonstrated the transfer of these labelled residues to the parasite.

There is no direct evidence for the protective role of these host antigens. However, studies using fluorescent antibody techniques and electron microscopy show that the young schistosomula have parasite antigens exposed on the surface, but have not acquired host antigens. In contrast, older schistosomula that have acquired a coating of host antigens no longer react with antiparasite serum. These older schistosomula are not damaged or destroyed by immune mechanisms *in vitro*.

In summary, antigens shared by host and parasite have been conclusively demonstrated in schistosomes. These antigens may be either of parasite or of host origin, and in spite of much circumstantial evidence it has yet to be shown conclusively that they serve to protect the parasite from immune attack. It is still a reasonable working hypothesis, however, that whereas antigens of parasite origin that cross-react with host antigens may serve to reduce the overall antigenicity of the parasite, it is those antigens of host origin that attach to the parasite surface that complete the immunological disguise of the adult worm and protect it from immune attack.

Possible mechanisms of immunological unresponsiveness

Four events can occur when the host comes in contact with an antigen. The host animal can produce antibody, can become primed without antibody synthesis, can develop cell-mediated sensitivity, or can become immunologically tolerant. In any contact with infectious agents one, several, or all of these processes may occur. In infectious diseases induction of even a partial state of tolerance could be detrimental to the host. Tolerance to schistosomes may result in healthy, long-living worms laying optimal numbers of eggs. Tolerance to the eggs would reduce the number of specific sensitized cells and/or antibody, which would result in the elimination or reduction of inflammatory cells and subsequent granuloma formation. Such tolerance may result from either central immunological unresponsiveness or peripheral inhibition. In central unresponsiveness there is an elimination of competent antigen-reactive cells, whereas in peripheral inhibition competent cells are present, but blocked. Blockage may be induced by over-reaction with the antigen, by specific suppressor T cells, or by antibody. Suppressor T cells may suppress either B cells or helper T cells. Examples of blockage are observed in tumour and tissue transplantation, where tolerance is the result of cytotoxic T lymphocytes being blocked by specific antibody, free antigen, or immune complexes. The possibility that specific or nonspecific complexes may block the interaction of the effector cells of antibody-dependent cell-mediated cytotoxicity with specific antigen should also be noted.

These regulatory mechanisms may be involved in schistosomiasis. A suppressed state may be the result of central unresponsiveness, antigen blockage, suppressor T cells, specific antibody inhibition, or inhibition by either specific or nonspecific antigen-antibody complexes. With central unresponsiveness it is most likely that the unresponsive state is due to tolerance of T cells in the presence of competent B cells. In this case, the unresponsive state can be terminated by by-passing the need for T cells with lipopolysaccharide or other agents. Similarly, attempts could be made to remove or inhibit the formation of suppressive immune complexes.

SUMMARY

Resistance to reinfectation by cercariae has been demonstrated in a number of animal species, and the resistance varies greatly from species to species. Resistance to reinfection can be measured by several methods including a recently developed rapid assay; this involves assessment of the number of schistosomula in the lung following cercarial infection compared with the number of adult worms recovered by perfusion several weeks after challenge. Other possible manifestations of immunity include the decrease in egg production by adult worms observed under certain circumstances, a phenomenon that might be exploited if the mechanism were understood, and the modulation of the host's reaction to the egg, a subject discussed in the section entitled *Egg granuloma* (p. 564). In normal infections the living adult worm is the major stimulus to protective immunity. Concomitant immunity, i.e., the presence of immunity to subsequent cercarial challenge in the continued presence of the living adult worm, represents one of the most intriguing problems in parasite immunology. The possibility of heterologous immunity deserves further study.

The mechanism of resistance is not completely understood. In rats and mice partial resistance can be transferred by serum; complement and unsensitized cells, possibly of two different types, have also been implicated in the rat. On the other hand, attempts to transfer resistance with sensitized cells have met with success only during the early stages of primary infection.

A number of different *in vitro* assays have been developed that are helping to separate the numerous mechanisms that might be involved. In the various models it has been reported that:

(1) Schistosomula can be killed by immune serum and complement (rat, hamster, monkey, baboon, and man). However, the antigen that appears to be involved, and that can absorb the lethal activity of the immune serum, does not render animals resistant when it is used as the immunogen.

(2) In a system comparable to (1), accelerated killing is obtained by a combination of antibody, complement, and polymorphonuclear leucocytes (rat).

(3) In a system in which damage to schistosomula can be quantitated by the release of ^{51}Cr from prelabelled larvae, damage is dependent on antibody and non-sensitized cells. The cells appear to be polymorphonuclear leukocytes, possibly eosinophils; complement does not appear to be involved (man, baboon). Sensitized lymphocytes do not produce damage in this system.

(4) Normal macrophages, sensitized with heat-

stable cytophilic antibody from immune rats, will damage schistosomula as assessed by electron microscopy. Development of this antibody coincides with the appearance and decline of immunity in the rat.

(5) Normal macrophages preincubated with immune serum for 3–14 h, but not washed, adhere to and damage schistosomula. The activity in the serum is heat-labile and has been shown to be an IgE antibody.

Evidence has been obtained that schistosomula acquire host antigens *in vivo*. This phenomenon has been analysed by studies *in vitro* that indicate that some host blood group substances, specifically those that are glycolipids, become incorporated into the surface of the schistosomulum and mask certain parasite antigens. The role of this phenomenon in protecting worms from immune attack remains to be clearly demonstrated.

The variety of immunological responses to schistosomal and egg antigens emphasizes the complexity of the immune response in general. Antigens stimulate both humoral and cellular responses. The humoral antibody response involves the production of multiple classes of antibodies with different biological functions that can have cytotoxic or suppressive properties; in conjunction with antigen they can form complexes that can themselves cause or prevent immune damage. The cellular response may also result in the production of sensitized cells that can manifest positive immune responses as well as suppressor activities. Many of these reactions occur simultaneously, and the final observed result may be only a small part of the total. When one adds to this the fact that there are multiple antigens which may elicit different responses, it becomes clear that the unravelling of the immune response in schistosomiasis will require much more work in order to define the antigens and mediator systems involved.

RECOMMENDATIONS

(1) Further studies on species variation in the development of resistance to reinfection are needed, with particular respect to the kinetics of induction, the response to different cercarial doses, and the differential effect of multiple versus single immunizing exposures.

(2) In rodent model systems the pathways and biological functions of complement should be analysed further. The class and antigenic specificity of the antibody that mediates passive transfer of immunity should be determined and related to the antibody involved in killing mechanisms *in vitro*. The nonspecific cells involved in immunity *in vivo* should be identified by use of specific anti-cell antibodies and adoptive transfer experiments when possible.

(3) The role of these mechanisms in other models, especially primates, should be investigated.

(4) Improvements in techniques for assaying resistance to reinfection, for example by using labelled cercariae, are needed.

(5) The timing and site of the effector event should be determined more accurately.

(6) The relevance of inflammatory lesions in the skin, lungs, and other possible sites to resistance to infection should be studied in more detail.

(7) The possible effect of augmentation of the number and function of nonspecific cell types, e.g., eosinophils, basophils, and macrophages, on resistance to infection should be examined.

(8) The possible advantage of using sites outside the hepatic portal system for the induction of immunity by adult worms should be tested.

(9) The effects of adjuvants (preferably biodegradable) in augmenting acquired resistance in experimental animals needs further study.

(10) In systems *in vitro*, studies are needed on the classes of antibody and types of cell involved, the target antigens (by absorption and inhibition reactions), and the role of each in the various animal model systems.

(11) The possibility of other mechanisms, especially activation of macrophages by IgE or lymphocyte mediators, should be investigated.

(12) Studies *in vitro* should be correlated with parameters of resistance *in vivo*.

(13) The role of host antigen in protection against immune attack *in vitro* needs further study, and experiments should be designed to test the role of host antigen *in vivo*.

(14) Further chemical characterization of host antigens is needed.

(15) The possible role of antigen turnover and/or modulation in the escape mechanism should be explored.

(16) Mechanisms of unresponsiveness should be evaluated, with particular respect to antigens which may play a role in resistance (central and peripheral

inhibition, including immune complexes and suppressor T cells).

(17) The effects on schistosomula of inflammatory products (pharmacological amines, lysosomal enzymes, etc.) should be tested.

(18) The immunological basis for worm pair sterility should be established.

(19) The role of basophil hypersensitivity *in vivo* and *in vitro* requires investigation.

(20) The role of cercarial penetrating enzymes in resistance to infection should be examined.

(21) Further attempts should be made to clarify the role of cell-mediated immunity, via sensitized lymphocytes, in resistance to infection.

(22) The following studies are important in the investigation of the response in man:

(*a*) Application of *in vitro* methods developed in animal models.

(*b*) Investigation of responses *in vitro* before and after treatment and testing of their relationship with the rate of reinfection.

(*c*) Studies on cross-reactions of serum from heterologous infections in tests *in vitro*, with special reference to swimmer's itch, the Formosan strain of *S. japonicum*, and other schistosomes affecting man and animals.

(*d*) Examination of the genetics of susceptibility to schistosome infection in man with particular relation to known Ir genes.

(*e*) Studies on the possible effects of schistosomiasis on other aspects of immunological responsiveness.

(*f*) Detailed evaluation of various immunological responses to defined schistosomal antigens.

(*g*) Testing for possible protective antibodies in man by heterologous transfer into experimental animals.

RELEVANCE OF ANIMAL MODELS TO MAN

Although numerous studies have been carried out on a variety of experimental animals infected with schistosomes and many data have been obtained on the possible mechanism of observed immunity and on the role of immunity in the development of pathological effects, the direct relevance of some of these findings to the development of immunological responses in man remains equivocal.

Although a number of animal species have been shown to be hosts of the schistosomes of man, the degree of infection tolerated by each host species varies considerably, and host specificity may be complicated by other factors such as intraspecific differences in susceptibility. Many experimental hosts of schistosomes can develop some level of resistance to reinfection, but there is marked variation in the level of immunity that develops and the speed with which it is acquired. There is a growing awareness of the need to define the criteria of acquired immunity more clearly since it is now generally considered that it may act in two phases: against the adult parasites of a primary infection and against immature stages of a second infection.

Many laboratory animals show innate immunity to the three common schistosomes of man. *S. japonicum* is the least host-specific, and the wide host range among domestic animals provides a variety of reservoir hosts. *S. haematobium* is much less adapted to nonhuman hosts, but a number of rodents and primates are susceptible and natural infections have been reported. The range of host specificity of *S. mansoni* is intermediate between those of the other two species, and natural or experimental infections are obtained in primates, rodents, insectivores, marsupials, and cattle.

S. mansoni has been used in most of the experimental infections carried out, and laboratory animals infected with this organism may be classified into 3 categories: those that fail to develop patent infections, those that develop transient infections, and those that become chronically infected. Primary schistosome infections may last for widely different periods in different host species. The rat loses most of its worms after 4 or 5 weeks, whereas in some primates a large proportion of the initial infection may persist for years. Other rodents, such as the guinea-pig and rabbit, undergo a form of " self-cure " that occurs at a slower rate than in the rat. Although the hamster and the mouse are both highly susceptible to infection with *S. mansoni*, most experimental work on acquired immunity has been done in the latter.

PRIMATE MODELS

Resistance to reinfection has been studied most intensively in the rhesus monkey, and studies on this

host have been described in *Resistance to infection in experimental animals* (p. 570). The rhesus monkey is clearly an excellent experimental animal for the study of both acquired immunity to *S. mansoni* and *S. japonicum* and its mechanism, but its relevance to man must be considered in relation to that of other primates. *Papio hamadryas*, *Cercopithecus aethiops*, and the chimpanzee do not show the dramatic fall in egg output that is characteristic of *S. mansoni* in the rhesus monkey. In this they more closely resemble man, in whom egg output following a primary infection may continue for a long period. It has been suggested that the baboon can develop partial immunity to *S. mansoni*, but the degree of acquired immunity that develops is generally far less than that of the rhesus monkey. It is clearly important to determine whether " concomitant immunity " occurs in other primates, particularly in those that have host-parasite relationships that are close to those between man and *S. mansoni* and between man and *S. haematobium*. The baboon is one of the better experimental models of human schistosomiasis and is a natural host of these parasites in Africa.

One species (*Papio anubis*) develops a strong acquired immunity to *S. haematobium* after a long period of immunization (100 weeks). This immune response appears to be " time-dependent ", since a challenge 27 weeks after immunization reveals only partial immunity with a 39% reduction of adult worms and no reduction of eggs deposited in the tissues. A challenge at 73 weeks, however, reveals a much higher degree of resistance that results in a worm reduction of about 80% and a reduction in tissue eggs of 96%. The observed low number of eggs deposited in the tissues of the immunized baboons indicates that, even though about 20% of the challenge infection becomes established, the adult worms have a greatly reduced egg-laying capacity. When such worms were transplanted from immunized animals into clean baboons, however, normal egg laying was resumed.

Studies on the development of " concomitant immunity " in the baboon have recently been undertaken by transplanting 50 worm pairs of *S. haematobium* from a baboon with a 16-week-old infection into clean baboons that were each challenged 35 weeks later with 7 000 *S. haematobium* cercariae. A reduction in adult worms of about 40% was obtained, but there was no reduction in tissue geg counts. The gross pathology of the immunized animals observed at autopsy was, however, very greatly reduced compared with that in the challenge control animals, and acquired immunity evidently reduced host reactions to the egg and, therefore, the development of pathological effects. Attention has already been drawn (see *Suppression of granuloma formation*, p. 565) to a possible alteration in immunopathological responses on second and subsequent exposures that may afford protection through the suppression of granuloma formation, possibly by the development of blocking antibodies.

Comment

The development of *S. haematobium* infection in experimental animals is deficient when compared to that of *S. mansoni* and *S. japonicum*. None of the small laboratory animals (mainly rodents) develop ideal infections, because most adult worms localize in the mesenteric rather than the vesical veins and viable egg output is low. Of these animals, however, hamsters appear to be the best experimental hosts. Among primates, *S. haematobium* infections have been studied largely in baboons and chimpanzees. Although the chimpanzee appears to be the better host, its limited availability suggests that the baboon is at present the animal of choice for studies of *S. haematobium*.

HETEROLOGOUS IMMUNITY

Immunity can develop across the species barrier (see *Resistance to infection in experimental animals*, p. 570). In calves, immunity to *S. mattheei* will develop following exposure to *S. mansoni*, and in mice and rhesus monkeys immunity to *S. mansoni* will develop after exposure to *S. mattheei* or *S. bovis*. These findings and others have led to the suggestion that exposure to animal schistosomes in some parts of Africa may diminish the susceptibility of the populations at risk to *S. mansoni* and *S. haematobium*.

The current interest in developing a human vaccine against *S. mansoni* raises the question of whether cross-immunity exists between *S. mansoni* and *S. haematobium*. Direct evidence of cross-immunity is very slight. Infections of both *S. mansoni* and *S. haematobium* commonly coexist in man, but this is not conclusive evidence for the absence of partial cross-immunity. Serum from baboons and man infected with *S. haematobium* exerts a marked lethal effect on schistosomula of the same species, but shows little or no cross-reaction with *S. mansoni* schistosomula. This is also true of the reverse situation. Subsequent studies *in vivo* in the golden ham-

ster, however, have shown that animals immunized by infections with *S. mansoni* or *S. haematobium* lasting 6 weeks, and challenged with the heterologous species, exhibit a considerable degree of cross-immunity in lung recovery and perfusion assays.

SUMMARY

The relevance of animal models to immunological responses in man relate: firstly to resistance to reinfection on exposure to both homologous and heterologous organisms, killed organisms, and their extracts; secondly to immunopathological lesions, especially inflammation and fibrosis around schistosome eggs trapped in the tissues; thirdly to the development of both antibodies and cellular reactivity; and fourthly to the detection and characterization of circulating parasite antigens. Although there is marked variability in the development of resistance to reinfection among experimental animals, much has been learned about the mechanisms involved in several mammalian hosts, in particular the rhesus monkey, the baboon, the rat, the mouse, and (most recently) the hamster. It should be noted, however, that most hosts develop only partial resistance. Techniques *in vitro* involving cultivation of schistosomula have added an extremely valuable experimental system. Immunopathological studies, devoted largely to granuloma formation around schistosome eggs, show similar responses in virtually all experimental animals studied, and these appear to be similar to those in man. Natural suppressor mechanisms also appear to occur in most hosts studied, including man. A wide variety of antibodies and cell-mediated reactions (both *in vivo* and *in vitro*) similar to those seen in man have been demonstrated in experimental animals. The presence of circulating antigens was first, but questionably, described in man. Recent demonstrations of circulating antigens in experimental animals should stimulate further studies in man.

RECOMMENDATIONS

(1) Further passive transfer studies in immunity and immunopathology are needed.

(2) Further studies on the effect of the duration and intensity of infection on the development and modulation of immunity and immunopathology should be carried out.

(3) Further studies are needed on the apparent suppression of egg laying in immunized primates and the determination of whether a similar phenomenon occurs in man.

(4) The relationship of humoral and cellular immunological parameters to the development of immunity and immunopathology should be investigated.

(5) Further studies of animal schistosomes within their natural hosts, and of heterologous infections, are desirable.

IMMUNITY IN MAN

Although experiments with different animals demonstrate the development of different degrees of immunity, these results have been obtained with numbers of cercariae (both in the immunizing and challenge exposures) far greater than those to which it is believed a human population is normally exposed. The proportion of infected snails in most habitats is usually low (<1%), though occasionally very high (50%) infection rates may be found. In most endemic areas transmission is seasonal and depends on the climatic conditions.

AGE PREVALENCE AND INTENSITY OF INFECTION

In the majority of endemic areas point or age prevalence rates of infection increase to a peak, usually in the second decade of life, and then decline. This is a finding common to the three main human infections. The extent of the decline in prevalence in the older age groups varies in different endemic areas and in all three species, and is possibly greatest in *S. haematobium*; this is thought by some to reflect different immunological responses.

The intensity of infection (as measured by the mean egg output in stools or urine of the groups examined) also varies with age and shows a similar pattern to prevalence.

The fall in prevalence and intensity of infection in adults is attributed by some to resistance to reinfection and to the gradual spontaneous death of the worm burden acquired during childhood. Quantitative water contact studies in Puerto Rico, Egypt,

Southern Rhodesia, and St Lucia, however, all show that adults have less contact than children with water; the fall in prevalence and intensity of infection may therefore be due to this diminished water contact, combined with the spontaneous death of adult worms.

The water contact of the two sexes may also differ. In Sierra Leone adult females have been shown to have a high degree of contact with water, and this is associated with only a slight fall in prevalence from the peak; on the other hand adult males have little contact with water and this leads to a considerable fall in prevalence.

In areas of very high *S. haematobium* transmission in Sierra Leone and Southern Rhodesia, the peak of prevalence is reached before the age of 10 years and declines thereafter. In nearby villages where transmission is less, the peak occurs during the second decade of life. It is unlikely that children in the high transmission areas over the age of 10 years have less contact with water than children of a similar age in the areas of lower transmission, and it is thought that the lower prevalence in the second decade of life in the high transmission areas is a result of suppression of egg output by some immune mechanism.

The egg output in different age groups in areas of high and low prevalence in Southern Rhodesia and St Lucia have been compared. Among children egg output is directly related to prevalence (i.e., high egg output occurs in areas of high prevalence), but among adults egg output is much reduced and is similar in areas of high and low transmission. This suggests that the mechanism suppressing egg output in adults may be more effective when high egg loads had been obtained in childhood.

A focus of *S. mansoni* in Uganda has been described, where " most boys are infected at the age of 3 years and most girls by 5 years. Thereafter the prevalence is effectively 100% in both sexes until death ". Peak output of eggs is in those aged 10–14 years (nearly 1 500 eggs per g of faeces in males, nearly 1 000 eggs/g in females), and whereas the female output drops to about half the peak level, in males there is only a slight drop in the older age groups. From this study there was little evidence of immunity in males except that the egg output did not continue to rise throughout life. The fall in egg output in females does suggest immunity. The pattern of prevalence and intensity of infection in this endemic area is very different from that of many other areas and suggests that any resistance that might develop to *S. mansoni* can be overcome.

It was suggested in 1952 that the most favourable conditions for maximum protection are moderate initial infection followed later by regular " booster doses " such as may be incurred by occasional exposure during one transmission period, and followed by an interval lasting to the next transmission season. " . . . the worst possible combination of factors is easy to imagine—heavy initial infections with increasingly stronger subsequent ones, together with exposure at practically any time of the year . . . " Others have come to a similar conclusion. These " worst " conditions may well prevail in the West Nile area of Uganda.

ADULT IMMIGRANTS TO ENDEMIC AREAS

An unusual type of survey was carried out in Brazil in a group of male adults who had moved from a nonendemic to an endemic area of *S. mansoni*. Prevalence and intensity of infection rose to a peak after 15–19 years of residence in the area and then fell, i.e., a pattern occurred similar to that among children brought up in an endemic area. This supports the view that the fall in prevalence after the usual peak in those aged 15–19 years is due to slowly acquired immunity rather than to reduced contact with water.

LONGITUDINAL STUDIES

In an investigation in the United Republic of Tanzania, in which standardized times for the collection of urine samples from 15 children were used for 3 years, it was claimed that *S. haematobium* egg output remained stable and therefore, since these children were being continually reexposed to infection, that they must have been resisting superinfection. However, it has been pointed out that when transmission was high in the first year of the study, egg output increased in 6 out of 7 heavily infected persons but increased in only 3 out of 8 lightly infected children, and a fall in egg count the following year could have been due to the reported reduced transmission.

CANAL CLEANERS

In a preliminary report on the excretion of *S. mansoni* eggs among canal cleaners in the Sudan (work that involved a high degree of contact with infected water) the egg output of workers who had been working in the canal for a few years only was very high, whereas among those who had been working for more than 5 years egg output was very low. Although this suggests that resistance to reinfection

had developed in those who had been doing the work for 5 years, it should be noted that this developed in only a few persons, since " some people had died apparently as a result of the disease, some fell very ill and were not able to keep their jobs ".

ATTEMPTED INFECTION OF HUMAN VOLUNTEERS

The classical study in this field [a] involved the infection of 6 volunteer fishermen aged between 35 and 45 years whose stools failed to show eggs of *S. intercalatum*, which was endemic in the area. After exposure to cercariae of *S. intercalatum* (now considered more usually a parasite of animals rather than man) stool examinations were negative at 3 and 6 months, but 3 men were passing a few eggs at 8 months. Mice exposed at the same time developed bisexual infections.

Gothe [b] exposed himself 16 times to cercariae of *S. haematobium* that had been treated with a 1 : 100 000 concentration of 20% wettable DDT powder. Subsequently he waded for 10 min in a stream known to contain infected *B. truncatus* and exposed himself in the laboratory to cercariae. Other than a transient skin irritation he had no signs or symptoms of infection and never passed *S. haematobium* eggs.

In Southern Rhodesia [c] two volunteers of European stock were exposed by immersion of the forearm and elbow for an hour. Simultaneously, two baboons were exposed to the same batch of cercariae obtained from infected *B. globosus* collected from the field. Neither of the volunteers passed eggs of *S. haematobium*. One developed a positive skin test and eosinophilia and one subsequently acquired an *S. mansoni* infection. The baboons were later found to have *S. mattheei*, a schistosome of domestic animals.

SUMMARY

It is very probable that immunity is not a controlling factor when the prevalence and intensity of schistosomiasis are light. In areas where infection is moderate to severe, some immunity appears to develop in the older age groups, in which the greatest morbidity occurs. In areas of extreme transmission it is possible that tolerance to the parasite may develop that prevents the development of resistance to reinfection.

RECOMMENDATIONS

(1) Additional carefully designed and controlled comprehensive epidemiological studies of prevalence, incidence, and intensity of infection in endemic areas are needed.

(2) Reinfection patterns after chemotherapy should be examined among patients living in endemic areas. Special attention should be paid to their relationship to those who are cured and to those with continuing low levels of infection after treatment.

(3) Studies on the longevity of worms in populations that have moved to nonendemic areas or after highly effective control programmes should be carried out.

(4) Further studies should be made on the passive transfer of antibody in man.

DIAGNOSIS OF SCHISTOSOMIASIS

Accurate and sensitive diagnostic techniques may be required not only for studies on individual patients but also for epidemiological surveys on groups of patients and for determining the effectiveness of chemotherapy and other control measures. A parasitological diagnosis based on microscopical examination of faeces or urine for the presence of the parasite (egg or miracidium) is a specific observation which allows no other interpretation. In contrast, none of the immunodiagnostic techniques so far developed for routine use are entirely specific. This lack of specificity may be overcome by the use of highly purified antigens, as discussed in *Recent advances in immunodiagnosis* (p. 587).

PARASITOLOGICAL DIAGNOSIS

Qualitative diagnostic techniques

Qualitative techniques are important in routine clinical practice and in establishing the endemicity of schistosomiasis, particularly in those areas where laboratory facilities are relatively primitive.

[a] Fisher, A. C. (1934) *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **28**: 227-306.

[b] Gothe, K. M. (1963) *Zeitschrift für Tropenmedizin und Parasitologie*, **14**: 512-518.

[c] Clarke, V. de V. (1966) *Central African journal of medicine*, **12**: 1-3.

For *S. haematobium* the simplest and most widely used method involves sedimentation and microscopical examination of a sample of urine taken near midday. Viability of eggs can be easily assessed, and diagnosis is both specific and extremely sensitive. Sedimentation is basically the simplest form of concentration, and the absolute efficiency of egg recovery from a sample is very high.

For *S. mansoni* and *S. japonicum* the direct faecal smear technique is still in widespread use at the clinic level. Its sensitivity is low and, although it may be useful in demonstrating eggs in trematode dysenteric syndromes or in high intensity infections, a concentration method is preferable for moderate or light infections or for infections of long duration. The simplest concentration method, and one of the most dependable for field diagnosis, involves comminution of the stools in 0.5% glycerinated water, followed by sieving and repeated washing and sedimentation. Refinements, including the formol-ether and the merthiolate-iodine-formol concentration techniques, depend on the removal of faecal detritus, mucus, and fats with acid and ether. The efficiency of egg recovery with such techniques is between 60% and 95%.

Hatching of miracidia from eggs is a simple and sensitive method for the demonstration of *S. haematobium* eggs in urine. The sample is diluted with clean, filtered water and exposed to light, and the miracidia can be seen with the naked eye. Miracidial hatching is less easy to achieve in *S. mansoni* and *S. japonicum* infections, and involves repeated washing and sedimentation of stools, followed by exposure to light.

All of the three common human schistosome infections may also be diagnosed by rectal biopsy. Egg viability may be determined by examination of a squashed preparation of the biopsy material after soaking for at least 15 min in water. In *S. haematobium* infections the great majority of eggs are dead and are coloured black, but in *S. mansoni* and *S. japonicum* infections viable eggs at different stages of maturity are commonly seen. The value of this technique lies in the isolation of viable schistosome eggs even when the stools are negative, and in the determination of the effectiveness of chemotherapy. After chemotherapy there is a change in the proportions of dead eggs and immature and mature living eggs found in the biopsy material.

Quantitative diagnostic techniques

Although qualitative techniques are satisfactory for routine clinical use, quantitative estimates of egg output are required in many areas of research. These include studies on epidemiology, on the dynamics of transmission, on most aspects of chemotherapy, and on the relationship between egg output and the prevalence and intensity of infection on the one hand and morbidity on the other.

A simple technique for *S. haematobium* that depends on the egg count in a 60-mm^3 subsample of a 10-ml urine specimen taken from a total urine collection is satisfactory for epidemiologic studies. More elaborate methods involve the filtration of urine and staining with ninhydrin, and the latest variant of this technique is currently undergoing intensive testing in a project on Lake Volta. A further technique that is useful for the monitoring of chemotherapeutic drugs involves quantitation of the hatching of *S. haematobium* eggs. This method, which gives both an absolute count and an estimate of the ratio of live miracidia to dead eggs, is highly sensitive. It also demonstrates that dead eggs may be shed in the urine even after successful chemotherapy.

Various improvements in the techniques for faecal examination in *S. mansoni* and *S. japonicum* infections, based on improved sieve design, have recently been introduced. These variants need further testing, however, and at present enumeration of eggs in these infections is based either on the Bell filtration-ninhydrin staining method or on the Kato technique. The latter was originally developed for studies in ascariasis and offers several advantages, namely, direct faecal sampling, an adequate sample size, rapid clearance of faecal background with a glycerin-malachite green solution, and diagnostic accuracy for many helminths. It can be used in the field and is therefore suitable for epidemiological survey work.

Summary

Quantitative diagnostic techniques have proved valuable in descriptive epidemiology, in pre- and post-control work in which the primary measurements of effect are prevalence, incidence, and intensity of infection, and in all aspects of applied pathological and chemotherapeutic studies. Although there is disagreement as to the best technique, which may differ with the situation, and on the sensitivity levels reached, such techniques will undoubtedly remain in use and will prove of great value in both clinical and experimental studies on chemotherapy, immunology, and control. Qualitative techniques will retain their importance in both individual and epidemiologic diagnosis and will complement other methods. It is important within any

one laboratory that the sensitivity of these techniques should be tested and a system of quality control maintained.

Recommendations

(1) In view of the importance of qualitative parasitological techniques in individual and epidemiological diagnosis, the sensitivity and acceptability of such techniques should be improved.

(2) Similar improvements in the sensitivity of quantitative techniques are needed for more accurate detection of low egg-output densities.

(3) To increase the efficiency of diagnosis, particularly when large numbers of people are involved, automated methods of egg counting should be investigated further. Possible devices include electronic cell counting systems and particle sizing computers.

(4) Interlaboratory studies of egg recovery, by means of both standard techniques and their new variants, should be conducted in order to increase knowledge of the degree of comparability currently achieved. Standardized techniques would provide more precision in inter-country comparisons than is available at present.

IMMUNODIAGNOSIS

Classical immunodiagnostic techniques

Two previous reviews have drawn attention to the multiplicity of techniques available for immunodiagnosis. These include: immediate and delayed intradermal tests using different stages of the schistosome as antigen; cercarial agglutination tests and the cercarienhüllen reaction; the miracidial immobilization test; several tests involving complement fixation, haemagglutination, flocculation, immunodiffusion, and immunoelectrophoresis; circumoval precipitin tests; and several varieties of fluorescent antibody test. With regard to these various tests, it has been pointed out that

" In judging the value of immunological methods, it should be borne in mind that parasitological demonstration of infection is a definite diagnosis, whereas an immunological diagnosis is based on indirect evidence (antibodies formed against the parasite). For this reason immunological methods should be used in practice only when the techniques are highly sensitive (significantly more so than coprological or urine examination), very specific, and carefully evaluated ".

As will be discussed later, the main difficulty about using such tests in the field has been their lack of sensitivity and specificity, which may reflect the crude nature of the antigen preparations that have been used. In addition, the cost of immunodiagnostic techniques is usually much greater than that of parasitological techniques, and the equipment required is more extensive.

Many comparisons of the various immunodiagnostic methods available have been carried out in field trials, and may be summarized as follows:

(1) The plasma card test and an intradermal test using the Melcher type ASP antigen have been found to be insufficiently reliable both for estimating prevalence rates and for the individual diagnosis of *S. haematobium* infection in the island of Zanzibar.

(2) Evaluation of indirect haemagglutination, fluorescent antibody, complement fixation, and charcoal test card techniques indicates that these tests are less satisfactory than a standard cholesterol-lecithin slide flocculation test.

(3) A bentonite flocculation test (the antigen being *S. mansoni* cercariae adsorbed onto bentonite particles) has been compared with a cholesterol-lecithin test, a complement-fixation test, an indirect haemagglutination test, and a fluorescent antibody test on living cercariae. None of the tests proved to be acceptably sensitive. In addition, cross-reactions in the bentonite flocculation test occurred in serum from patients with trichinosis, visceral larva migrans, syphilis, echinococcosis, filariasis, and ascariasis, as well as in some samples of normal serum. The specificity of the bentonite flocculation test was therefore too low for routine use, and the sensitivity of the other tests made them of doubtful value.

(4) Extensive field trials on immunodiagnosis of *S. mansoni* infection have also been carried out in the Caribbean. Five different antigens (*S. mansoni* cercariae, adult *S. mansoni*, and eggs of *S. mansoni*, *S. haematobium*, and *S. japonicum*) were evaluated by complement-fixation, cholesterol-lecithin flocculation, and fluorescent antibody techniques, as well as by their ability to elicit immediate and delayed skin reactions. Patients tested included inhabitants of St Lucia infected with *S. mansoni*, uninfected people from St Vincent, and Negroes from Cleveland, Ohio, USA. A large number of patients with proven *S. mansoni* infection failed to show an immediate skin reaction, while many false positive reactions, both immediate and delayed, were seen in uninfected individuals. Of the serological techniques, the fluorescent antibody test proved more sensitive than the cholesterol-lecithin or complement-fixation tests, but

a high proportion of false negatives occurred in all age groups. Specificity of the serological tests was poor, and large numbers of false positive results were observed. These may be attributable to cross-reactions between the three common species of schistosome affecting man and other mammalian and avian schistosomes.

(5) Immediate and delayed skin reactions after intradermal injection of *S. mansoni* adult worm and cercarial antigens have been evaluated in Uganda. The sensitivity of the immediate skin reaction was high in subsamples of the population, and adult worm antigen was superior to cercarial antigen, but the specificity of this test was low. The sensitivity of the delayed skin test was unacceptably low.

(6) Intradermal tests using an antigen from *S. japonicum* have proved sensitive in known infected individuals in the Philippines, but some false positives occurred in uninfected subjects while large numbers of patients infected with *Capillaria philippensis* gave positive reactions.

(7) International comparisons of the sensitivity and specificity of serological tests have been carried out on populations in Chad and Ethiopia (endemic areas) and in Peru and Afghanistan (nonendemic areas). Slide flocculation with cercarial antigen of *S. mansoni* was highly sensitive but poorly specific. Complement fixation using adult worm antigen, in contrast, was reasonably specific but poorly sensitive, and some samples of serum from patients with ascariasis, trichinosis, and echinococcosis gave false positive results.

These various trials have clearly indicated that none of the immunodiagnostic techniques in routine use at present offers a satisfactory alternative to parasitological diagnosis. The tests are not sufficiently sensitive in detecting infected individuals and, more important, they are also not sufficiently specific. This presumably reflects the extremely crude nature of the antigens that are used.

Recent advances in immunodiagnosis

The conclusions reached in the last section indicate that the value of an immunodiagnostic technique for schistosomiasis rests on several criteria. Most important, it should be both specific and sensitive. It should also be quantitative and applicable to mass surveys. Finally, its value would be enhanced if it helped to identify active infection or the success of chemotherapy, and if it could yield information on the immune status of the host.

It has already been indicated that one of the main reasons for the lack of specificity of the currently available immunodiagnostic techniques is the impurity of the schistosome extracts that are used as antigen. The preparation of pure antigen involves long, highly sophisticated, and therefore costly procedures. Such antigen is unsuitable for use in the classical immunodiagnostic techniques since, except for intradermal reactions, these are of low immunologic sensitivity and would require large amounts of antigen. Two developments are therefore required for the improvement of immunodiagnostic tests: firstly the preparation of pure antigen, and secondly the development of tests that use only very small amounts of antigen.

Recent progress has been made in both of these fields. Affinity chromatography, using either specific enzyme inhibitors or immunosorbents, now makes it possible to produce reasonable quantities of highly purified antigen in a relatively simple way. Three schistosomal antigens have been prepared by this method: acetylcholinesterase, a genus-specific antigen, and a species-specific antigen from *S. mansoni*. In addition, three highly sensitive techniques are now available for detecting antibodies to very small amounts of these antigens:

(1) *Radioimmunoassay* is a useful technique, but is limited to highly equipped laboratories.

(2) The *defined antigen substrate sphere* (DASS) method has recently been applied to schistosomiasis. In this technique *S. mansoni* antigens are covalently bound to agarose beads that serve as a matrix for an immunofluorescence reaction with serum from infected individuals. The main advantage of this technique, when compared with the classical fluorescent antibody test, is that it is possible to make rapid measurements of fluorescence by automated microfluorometry.

(3) The *enzyme-linked immunosorbent assay* (ELISA) has recently been applied to the diagnosis of parasite infection, initially in trichinosis and hydatidosis and more recently in other conditions. In this system small amounts of the antigen are coated onto polystyrene tubes and diluted serum is added. Noncomplexed immunoglobulin is washed out, and peroxidase-labelled anti-human Ig is added. The tubes are incubated, excess conjugate is washed out, and the amount of peroxidase fixed on the tubes is determined with hydrogen peroxide as the substrate and *o*-dianisidine (3,3′-dimethoxy-[1,1′-biphenyl]-4,4′-diamine) as the hydrogen donor. The amount of

enzyme fixed to the solid phase, as estimated by spectrophotometry, provides an exact measurement of the quantity of specific antibodies in the serum. The test has been applied to schistosomiasis, and specific antibodies (or their fragments) have been found both in the serum and in the urine of infected subjects.

The ELISA system appears to be a promising quantitative method that can be easily automated. High specificity can be achieved by the use of purified antigen, and since the method is also immunologically sensitive (requiring only 4 μg of antigen and 4 μl of serum diluted 1 : 500) it will almost certainly become a useful method in the future of epidemiologic immunodiagnosis.

The use of purified antigens and the techniques described above now make the specific and quantitative study of antibodies in schistosomiasis possible and particularly applicable, through automation, to mass surveys. Other approaches, however, have also to be considered, particularly for evaluating the evolution of the disease and the results of therapy. The detection of circulating antigens and soluble immune complexes has already been described in *Immune complexes* (p. 566). In this context, recent studies in Brazil have indicated the existence of two specific schistosome antigens in the urine of patients infected with *S. mansoni*. The presence of one of these antigens is correlated with the intensity of infection as estimated by egg output. Further studies are required to assess this observation as a new way of evaluating active *S. mansoni* infections and of determining the effects of chemotherapy.

Conclusions

Immunodiagnostic techniques currently available for routine use lack the accuracy, sensitivity, and specificity characteristic of parasitological methods. They can be used in epidemiological investigations or in individual patients as supplements to direct parasitological diagnosis, but are unsatisfactory when used on their own. The future of immunodiagnostic methods will depend on two developments: firstly the improvement of specificity by the use of purer antigen preparations, and secondly increased sensitivity of the assay methods and reduction in the amount of antigen required for testing. Progress at the research level has been made in both of these areas, and it is likely that the new techniques that have been developed will prove useful in field studies. It should be emphasized again that all of the available techniques are diagnostic only; there is no evidence of correlation between any serologic parameter and protective immunity.

Recommendations

Further studies should be carried out on:

(1) The purification of antigen, and the development of specific and quantitative serologic tests including radioimmunoassay.

(2) The development of sensitive and automated techniques requiring minimal amounts of antigen and serum.

(3) The evaluation of circulating and urinary antigens and their correlation with the state of infection.

GENERAL RECOMMENDATIONS

In parasitic diseases in general, immunity evolves very slowly and is associated with the persistence of viable organisms. This is in contrast to the immunity acquired in many bacterial and virus diseases. For this reason the possible development of immunophylaxis in parasitic diseases, including schistosomiasis, depends on detailed analysis of the host's immune responses. Much more basic work is required before practical objectives, such as immunoprophylaxis, mitigation of immunopathological lesions, and improved immunodiagnosis, can be achieved.

The essential pathology of schistosomiasis is a result of allergic reactions around eggs retained in the tissues. These reactions are similar in man and most experimental animals so far investigated. In addition, immune complexes deposited in tissues could play an important role in the pathogenesis of the disease. The following particular problems require further investigation:

(1) Biochemical and immunochemical characterization of materials secreted by eggs of the three schistosome species infecting man, and more detailed studies of the life-span of the eggs and their rates of destruction, are needed.

(2) The immunological mechanisms determining the formation of granulomas and their diminishing size during the later stages of the disease should be

investigated further. Attention should be given to the role of the eosinophil in these reactions, and to the possible involvement of IgE and immune complexes.

(3) Swimmer's itch, Katayama fever, and renal lesions should be investigated to elucidate their allergic mechanisms. Special attention should be given to the dynamics of immune complex formation and to the interactions of complement.

(4) The prevalence of hepatosplenic or urinary disease in populations, and the relationship of this to intensity of infection and the genetic constitution of the host, needs more detailed study.

In contrast to the immunopathology, protective immunity as determined in experimental animals is primarily induced by antigen from the adult worm. Furthermore, the extent and pattern of immunity differ strikingly among animal species. Surprisingly, there is still no certain evidence of immunity in man. Now that much information is available in experimental animals, special attention should be paid to the limitation of disease in man.

Particular problems requiring further investigation are:

A. *In man*

(1) Longevity of the worms.

(2) The incidence, prevalence, and intensity of infection in populations entering and resident in endemic areas, and in populations following mass treatment.

(3) The mechanisms of immune destruction, analysis of which requires improved assay systems *in vitro*. Such systems could establish the identity of the protective antigen and the respective roles of antibody of different classes, of complement and of effector cells, as well as the part played by immune complexes.

B. *In animals*, where the demonstrable immunity is directed primarily against reinfecting schistosomula, information is especially needed on:

(1) The precise route of migration of the schistosomulum.

(2) The exact sites and modes of destruction of the parasite.

(3) As in A. (3) above.

(4) Correlation of immunity *in vivo* with tests of worm damage or death *in vitro*. Mechanisms of immunity could also be elucidated by studies *in vivo* involving passive transfer and immunosuppression.

C. *Exploration* of the possible effects of immunity on reproductive processes of adult worms.

D. *Explanation* of the persistence in the blood stream of adult worms that do not possess a protective cuticle. Further work is required to establish the protective role of:

(1) Endogenously synthesized and acquired host antigens bound to the worm surface.

(2) Antigens secreted by the worms.

(3) Antigen-antibody complexes.

(4) Specific tolerance.

At present the definite diagnosis of schistosomiasis can be achieved only by the demonstration of parasite eggs in the excreta or in biopsy material. Improved immunological diagnosis requires isolation and characterization of species-specific antigens. These can be used not only for the detection of antibody but also for the development of sensitive assay techniques for antigen in body fluids. These tests could possibly provide a measure of the worm burden.

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Annex

STATISTICAL CONSIDERATIONS IN PARASITOLOGICAL DIAGNOSIS

In the detection of individuals who pass schistosome eggs in the excreta there is a bias towards an underestimation of the prevalence of positive cases. A false diagnosis of a negative case as positive does not occur, except through contamination or the faulty labelling of specimens.

In the overwhelming majority of infections the detection of schistosome eggs in the urine or stools presents no statistical problems. In light infections the probability of failure to detect a positive case increases, since the egg output may be very low. If this occurs no eggs may be found in small specimens or small samples. In this event several factors increase the probability of egg detection, as follows:

(1) The combination of extremely low excretal egg output and a specimen of low volume occurs only rarely. Therefore, taking into account the total frequency distributions of egg outputs and specimen volumes, this combination of adverse variables occurs in only a very small proportion of the totals possible.

(2) Repeated specimens of excreta and replicated sample examinations reduce the probability of failure to detect a low egg output to extremely low levels.

(3) Concentration techniques, which have a high absolute efficiency of egg recovery, greatly increase the probability of egg detection.

Tabulated probabilities of egg detection are based on the Poisson distribution with sampling from a specimen in which the distribution of eggs is made homogeneous. More detailed tabulations can be generated for specific sample volume sizes and expected egg outputs with different numbers of replicates, as has been done in hookworm infection.

Since the overdispersed parasite population in the vertebrate host assumes the form of a truncated negative binomial distribution, a histogram of the frequency distribution of excretal egg output in a population would be highly positively skewed. In practice this means that the probability of egg detection at low output levels assumes a perhaps disproportionate importance; at higher outputs there is an extremely low probability of failure to detect eggs by means of standard techniques, reinforced when necessary by examination of repeated specimens, replicated samples, and concentration techniques.

Although no parasitological technique is absolutely sensitive, since eggs may be lost at various stages of a technical manœuvre, the confidence placed in techniques of egg recovery appears justified. The main deficiency is in the single sample examination of low density infections with small excretal egg outputs, as in epidemiological and control field work. In control studies the effectiveness of intervention measures may be overestimated by failure to detect low egg outputs in a single examination. This may assume significance in determining whether or not transmission has ceased following application of a control measure. In correlative morbidity studies the failure to detect very low egg outputs is less significant, since the area of greatest interest is the total frequency distribution of egg output.