that where initial infection is heavy, as in irrigation farming, the Bantu population at risk may suffer grave illness and disability. Many parts of Africa will soon be faced with much heavier cercarial attacks and a concomitant increase in the severity of the disease in all races, because the key to the development of much of east, central and southern Africa lies in a greatly increased provision and use of water. This prospect and the present very high infection rate make it important that a combined investigation should be undertaken to arrive, if possible, at an accurate evaluation of the effect of the disease on health. Such an investigation cannot be carried out in conjunction with multifarious routine duties, and a full-time unit should include a physician, a pathologist well-versed in parasitology, a physiologist, and possibly also a psychologist. These workers should study uninfected and infected young Africans, and if a difference in health and performance can be demonstrated, they should then evaluate the effect of treatment on this difference. It is of great interest to note that accurate studies of this kind are now being carried out in the Union of South Africa under the aegis of the South African Council of Scientific and Industrial Research.

Acquired Resistance to *Schistosoma* Infection in Experimental Animals

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The question whether animals and human beings infected with *Schistosoma* can acquire resistance to reinfections or super-infections has only recently aroused general interest, although the possibility of such immunity was discussed by Fujinami in 1916,[a] and later by Ozawa,[b] Fairley, Macky & Jasudasan,[c] and Fisher.[d] The importance of the question is beyond doubt, since if it were proved that not only animals but also human beings develop effective resistance to schistosomes, current ideas on treatment might need modification. Indeed, it might even be possible to produce resistance artificially.

However, at the present time we are still far from being able to draw such conclusions. Our knowledge of acquired resistance to schistosomes, based as it is on animal experiments, is still very incomplete. As regards the resistance of human hosts, we have so far been forced to rely on conjectures based mainly on the age-distribution of cases of vesical bilhar-

[a] Fujinami, A. (1916) *Kyoto med. J.*, **13**, 176
[b] Ozawa, M. (1930) *J. exp. Med.*, **8**, 79
[c] Fairley, N. H., Macky, F. P. & Jasudasan, F. (1930) *Indian med. Res. Mem.*, **17**, 53
[d] Fisher, A. C. (1934) *Trans. Roy. Soc. trop. Med. Hyg.*, **28**, 277

ziasis, Oriental bilharziasis and *Schistosoma intercalatum* infection. As Fairley[e] and, more recently, Newsome[f] have mentioned, experimental reinfection of human volunteers is the only completely reliable method of demonstrating the presence or absence of this resistance in human beings. Once such proof is available, conclusions based on analogies between animals and human beings will be more justifiable than hitherto.

In the following, an attempt is made to give a general summary of the results of animal experiments, although it is not claimed that this treatment of the subject-matter is complete. In the following discussion, purely theoretical considerations are set aside in favour of reliable observations and of justifiable conclusions therefrom.

Mice, hamsters and monkeys have been used almost exclusively as experimental animals. The small rodents have the advantage that their use makes it possible to organize large experimental and control series which can be statistically analysed—a procedure which is usually impossible with monkeys. On the other hand, observations on monkeys are probably more suitable for drawing analogies with human beings, in so far as conclusions of this nature are regarded as at all permissible.

As mentioned in communications by various authors, mice and hamsters acquire a resistance to reinfection with *S. mansoni*, *S. japonicum*, and *Schistosomatium douthitti*. This resistance is revealed by the fact that in a second or subsequent infection fewer cercariae develop into the mature worms than after the initial infection, provided that the immunizing infection is allowed to act for a month or more. The experiments of Kagan[g] with *Schistosomatium douthitti* also showed that the worms developing in challenge infections were stunted in growth. Repeated infections seemed to produce a more intense resistance than a single infection. However, resistance was never so complete as in the case of many monkeys.

In monkey experiments it must be borne in mind that these animals are not equally good hosts for all species of *Schistosoma*, nor are they as good hosts as man for species which are peculiar to him. Infection of macacos with the ruminant parasite *S. spindale*, and with the mouse parasite *S. douthitti*, led only to a transient attack, and the young worms died within two to four weeks before any eggs were excreted.[c,h] On the other hand, *S. haematobium*, *S. mansoni* and *S. japonicum* remain alive in various monkey species for several months or years and produce many eggs. Nevertheless, even in these species there are differences in the length of life and in the duration of egg excretion, depending on the type of monkey used. For example, in the case of *S. mansoni* infection, long-tailed monkeys (*Cercopithecus aethiops*), according to my own observations, and baboons (*Papio hamadryas*), according to Newsome,[f] excrete eggs for several years—much longer than do rhesus monkeys (*Macaca mulatta*).

[e] Fairley, N. H. (1951) *Trans. Roy. Soc. trop. Med. Hyg.*, **45**, 279
[f] Newsome, J. (1956) *Trans. Roy. Soc. trop. Med. Hyg.*, **50**, 258
[g] Kagan, I. G. (1952) *J. infect. Dis.*, **91**, 147
[h] Kagan, I. G. (1953) *J. infect. Dis.*, **93**, 200

Cram & Files,[i] Standen,[j] and Meleney & Moore[k] have reported resistance acquired by rhesus monkeys to *S. mansoni* infections. According to all these observations, in infected rhesus monkeys the excretion of *S. mansoni* eggs ceases after several months or falls to a very low level. When monkeys in this stage are exposed to reinfection, the excretion of eggs is neither renewed nor increased. Meleney & Moore state that a rhesus monkey twice exposed, with an interval of 17 months, to infection with *S. haematobium* behaved in the same manner.

Newsome[f] reported that baboons (*Papio hamadryas*) retained an infection with *S. mansoni* for years. Reinfections with *S. mansoni* produced a resistance that enabled the monkeys, though heavily infected, to survive a dose of 100 000 cercariae, which is usually lethal to baboons that have not been infected.

Vogel & Minning[l] followed the course of the disease in rhesus monkeys which were repeatedly exposed to infection with *S. japonicum*. With the help of egg counts and miracidia hatching experiments, egg excretion was followed up for several months, as a rule even for several years. In *Macaca mulatta*, *S. japonicum* does not give rise merely to a transient attack. These experiments do not indicate how long egg excretion continues after the first infection, since most animals were repeatedly infected. In the case of one animal, egg excretion had not completely ceased almost two years after a single infection. The symptoms produced by this type of bilharziasis (*S. japonicum*) in the rhesus monkey are predominantly in the form of intestinal illness, and the extent of egg excretion in the faeces is roughly parallel to the severity of the disease. Initial infections with 800-1500 cercariae of both sexes caused fatal illnesses, accompanied by dysenteric symptoms and the excretion of large quantities of eggs. On the other hand, initial infections with 200-400 male and female cercariae were resisted by the monkeys without any noticeable falling-off in their general condition and were accompanied by a moderately high egg excretion.

I shall now give a few examples from these experiments. A rhesus monkey was infected with 100 male and 100 female cercariae. Thirty-seven days later, egg excretion commenced and continued for 10 months at a low level without any appreciable change. On the 273rd day after the first infection, the monkey was exposed to a second bisexual infection with 600 cercariae. Six weeks later this resulted in a steep rise in the egg counts and a fatal intestinal illness. Thus, 9 months after the first infection this animal had still not acquired any effective resistance against a strong superinfection.

Another monkey was infected with 259 cercariae of both sexes. Egg excretion, which commenced on the 40th day, was moderately high and continued for a long time with some fluctuations. From about the 250th day onwards, it began gradually to fall off, without completely ceasing. Barely a year after the first infection, the monkey was exposed to a second

[i] Cram, E. B. & Files, V. S. (1947) *Nat. Inst. Hlth Bull.*, **189**, 101
[j] Standen, O. D. (1949) *Ann. trop. Med. Parasit.*, **43**, 268
[k] Meleney, H. E. & Moore, D. V. (1954) *Exp. Parasit.*, **3**, 128
[l] Vogel, H. & Minning, W. (1953) *Z. Tropenmed. Parasit.*, **4**, 418

infection with 600 cercariae. Forty-four days later a new period of increased egg excretion commenced. However, although the number of cercariae was twice as high, this period was shorter than that following the first infection—an indication of an acquired partial resistance.

On the 471st day after the initial infection the monkey was infected for the third time, on this occasion with 1200 cercariae—a dose which would have been fatal for monkeys not previously infected. Nevertheless, in the case of this monkey, the strong infection resulted only in an increase of egg excretion lasting for a short time. This again indicated a partial resistance. Egg excretion then ceased, so that not only the egg counts but also the hatching experiments were negative.

After a fourth infection, again with over 1000 cercariae, which took place 21 months after the first infection, egg excretion ceased completely for the first time. Thus the fourth infection encountered a complete resistance, which afforded protection even against a dose normally fatal. Similar observations were made with other monkeys, which were also exposed to infection on several occasions separated by long intervals.

In the case of three more rhesus monkeys, a different experimental method was chosen. These animals were exposed to numerous, weak infections, closely following one another. At intervals of about one month we allowed, on each occasion, 25 cercariae, and later 60 or 100 cercariae, to penetrate the skin of the abdomen. This was repeated 13-17 times. During the whole series of experiments the egg excretion was followed up. As was to be expected, the egg count rose at the outset. However, this increase did not continue indefinitely, and 7-13 months after the first infection a fall in the egg count took place in all three monkeys. Although the monthly infections were continued and the number of cercariae increased, the number of eggs fell so low after some time that they could no longer be counted, and furthermore only isolated specimens of hatched-out miracidia could be detected. From this it could be concluded that about 9-12 months after the first infection the three animals had acquired a strong resistance to weak super-infections.

Some 14-18 months after the first infection, 1500 cercariae were placed on the skin of each of the three monkeys. The fact that this was not followed by further egg excretion indicated that the three monkeys were also protected against very strong infection.

A similar experiment with another rhesus monkey, which we had exposed to infection with 30-60 cercariae each month, gave a different result. Before the animal could build up an effective resistance it fell ill with severe intestinal bilharziasis, which was accompanied by high egg excretion and led to death.

From the examples given, as well as from the results of further experiments, it is clear that moderately strong bisexual infections with *Schistosoma japonicum*, which did not greatly affect the state of health, led in the case of *Macaca mulatta* first to a partial, and later to a complete, resistance against super-infection with the same parasites. This resistance is also effective against very high doses which would be fatal for normal monkeys. It develops relatively slowly. Partial resistance was observed in 5 monkeys,

304-886 days after the first infection and complete resistance in 10 monkeys 420-966 days after the first infection. Since the challenge infections took place at long intervals, it is possible that these degrees of resistance had already been reached at an earlier stage.

On dissecting monkeys which had shown complete resistance to superinfection with *S. japonicum*, we always found male and female worms, usually in small numbers. These worms were somewhat smaller than normal and the egg production of the females was apparently greatly decreased, so that eggs were no longer, or only very rarely, detectable in the faeces. It would be of considerable theoretical, and possibly also of practical, interest to know whether these surviving schistosomes are necessary for the maintenance of resistance—i.e., whether the monkeys exhibit a state of protection which should be regarded as premunition. If this is the case, then resistant animals, on losing their schistosomes or on being freed from them by drugs, should be susceptible to fresh infection. In order to find the answer to this question we subjected a rhesus monkey, which had been found to be completely resistant after four infections with *Schistosoma japonicum* and passed no more eggs, to intensive treatment with tartar emetic with the aim of killing off any surviving worms. In the years which followed, the monkey was exposed to seven strong challenge infections, the last taking place six-and-a-half years after treatment. All these infections encountered complete resistance, for in 315 samples of faeces taken throughout this period, neither an egg nor a miracidium was discovered. Furthermore, during the final dissection, neither schistosomes nor eggs were found. In the case of this animal, therefore, there seemed to be a genuine immunity, independent of surviving worms, which persisted for years.

Kagan & Lee[m] came to another conclusion in experiments on mice infected with *Schistosomatium douthitti*. They found that the acquired immunity ceased about three weeks after the end of an effective treatment. However, the immunizing infection in these experiments had only been allowed to act for 31-71 days, whereas in our rhesus monkeys the corresponding period was at least 600 days. The time factor appears to play an important role in the building-up of resistance to schistosomes. Further observations relating to the important question as to whether we are dealing with premunition or genuine immunity are very necessary. New experiments we are carrying out with five monkeys for this purpose are unfortunately not yet completed.

Olivier & Schneidermann,[n] as well as Vogel & Minning,[l] made an attempt to determine the fate of immature forms of *S. mansoni* and *S. japonicum* which had penetrated into resistant mice or apes. Their findings indicated that a large proportion of the young parasites passed through the skin and were then destroyed in the schistosomulum stage, either while travelling to the lungs or in the lungs themselves. Vogel & Minning found in sections of the lungs of resistant monkeys, which had been exposed 4-7 days before death to large numbers of *S. japonicum* cercariae, numerous small foci of inflammatory cells, which surrounded the schistosomula. In this

[m] Kagan, I. G. & Lee, C.-L. (1953) *J. infect. Dis.*, **92**, 52

[n] Olivier, L. & Schneidermann, M. (1953) *Amer. J. trop. Med. Hyg.*, **2**, 298

experiment the portal vein branches were reached only by a few schistosomula.

A further problem calling for clarification concerns the nature of the stimuli which set in action the defence mechanism against schistosomes. From the theoretical viewpoint such stimuli could arise either from the various stages of development of the worms or from the eggs. Perhaps the organic matter from dead schistosomes or dead eggs plays an important role; perhaps, on the other hand, the essential stimuli are provided by the excreta of living worms or eggs. It must also be borne in mind that several of the factors mentioned produce the same effect.

If the organic matter from dead schistosomes supplies immunizing stimuli, then it would seem to follow that immunization can be induced artificially by injection of such matter. So far animal experiments along these lines have given contradictory results. Ozawa[b] believed that he could detect a partial resistance to *S. japonicum* infection in dogs after he had injected matter from *Schistosoma* worms or cercariae into the animals. Lin, Ritchie & Hunter[o] carried out experiments with *S. japonicum* on mice, and found that after eight injections of a *Schistosoma* antigen the animals developed fewer worms in challenge infections than did the corresponding infected control mice. My own attempts to immunize two rhesus monkeys against *S. japonicum* infection with the aid of a " vaccine " were unsuccessful. Although the fresh or dried organic matter from over 2000 male and female schistosomes was administered to both animals in 10 or 12 injections, challenge infections with 300 to 400 cercariae resulted in a prolonged and in part heavy excretion of eggs. Thus there was no recognizable sign of resistance.

Kagan[g] presumed that the eggs play an important part in giving rise to resistance, because in mice infected with *Schistosomatium douthitti* resistance set in simultaneously with the commencement of egg production. Vogel & Minning[l] attempted to ascertain whether infections in which no eggs occurred could also give rise to resistance. They exposed two rhesus monkeys 10 or 12 times to infection with exclusively male *S. japonicum* cercariae. The total number of cercariae employed was 2400 and 1628 respectively. Such monosexual infections have the great advantage that they do not noticeably impair the state of health of the animals, even in the case of heavy infections. The two monkeys were infected with 400-500 cercariae of both sexes 23 and 20 months respectively after the first exposure. In one animal this was followed by a noticeably short period of egg excretion, while in the other, eggs were almost completely absent from the faeces. These results seem to speak in favour of a moderate to high degree of partial resistance. However, they were not confirmed by Meleney & Moore.[p] These authors infected two monkeys (*Macaca mulatta*), one with male worms of *S. mansoni* and the other with female worms. Subsequently, each was infected with worms of the other sex. Both animals developed egg passage after the normal prepatent interval, and at autopsy normal adult worms were recovered. The two experiments of Meleney & Moore differ from ours in that the

[o] Lin, S., Ritchie, L. S. & Hunter, G. W. (1954) *J. Parasit.*, **40** (Suppl.), 42

[p] Meleney, H. E. & Moore, D. V. (1954) *Exp. Parasit.*, **3**, 128

monosexual infections had only acted for 11 or 12 weeks as against 20 or 23 months in our experiments.

We deemed it advisable to repeat our experiments with monosexually infected monkeys and I am now in a position to give a summary report on four new experiments of this nature.

Four monkeys (*Macaca mulatta*), three males and one female, were repeatedly infected with male *S. japonicum* cercariae. The three male monkeys were exposed to two or three infections with a total of 1500-3000 cercariae. The female was infected 12 times with, in all, 4700 cercariae. All four monkeys were exposed to bisexual infection with 400-500 *S. japonicum* cercariae, 1½-7 months after the last monosexual infection. In the following 3 months, examinations of the faeces for eggs and miracidia were negative in all four animals. Next we placed about 1000 cercariae of both sexes on the skin of the four animals. In the case of the three male monkeys, despite numerous examinations, we could not detect a single egg or hatched-out miracidium in the following eight months. However, the female began 33 days after the last strong infection to excrete *S. japonicum* eggs. In the next few days her abdomen became very prominent and the skin took on an unhealthy pale yellow colour. About two weeks later the female monkey was found one morning lying very ill and in a highly anaemic state on the floor of the cage, and to our surprise there was a stillborn young one beside her. The animal then slowly recovered. The egg excretion, for which the figures were high for a short time, ceased completely 2½ months after commencement and had not recommenced in the course of 5 months.

These experiments are instructive from two different points of view. They show that monosexual infections with *S. japonicum* may lead to complete resistance to bisexual infections in rhesus monkeys. Furthermore, the course of the experiment in the female shows the lability of this resistance. A pronounced weakening of the host, caused in this case by gravidity and anaemia, may bring about the collapse of resistance.

Studies on Parasitism and the Nutritional State *

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The subject of parasitism and the nutritional state is one of considerable significance to non-white populations, particularly those dwelling in the tropics and semi-tropics, yet it has attracted relatively little serious attention. Consequently, the field is pervaded with speculation and differences of opinion.

For some time past, I have been collaborating with several other workers at the South African Institute for Medical Research in studies

* These studies were carried out in collaboration with the Bilharzia Natural History Unit (Hon. Director Dr B. de Meillon) of the South African Council for Scientific and Industrial Research.