The Relationship between Acquired Resistance and Transmission of *Schistosoma* Weinland, 1858, in Man, and its Influences on the Prevalence of *S. capense* (Harley, 1864) and *S. mansoni* Sambon, 1907, in Southern Rhodesia.

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INTRODUCTION.

Bilharziasis ranks with Tuberculosis and Malaria as the three most important endemic diseases of man in Rhodesia. The prominence given to bilharziasis in the endemic diseases pattern arises from its high prevalence, particularly in the African population.

It is probable that bilharziasis was endemic in the country even prior to the arrival in the country of the European settlers in 1890, but it was not until Orpen (1915) described the results of a small survey that local infections were proven. He reported 182 (31 per-cent) urinary infections in 592 African prisoners examined in the Salisbury gaol. In the ensuing 15 years there was only scanty evidence of the prevalence of the disease. However, in the decade 1931 to 1940 the Annual Public Health Reports of Southern Rhodesia indicated increasing prevalence, and this stimulated interest in the problem and led to the establishment in 1939 of a specialised laboratory to study the parasites causing the disease. The unpublished records of this laboratory indicate that in more recent years the disease has increased not only in prevalence but also in its intensity in infected individuals. Surveys show that the urinary form of the disease is more widespread than the intestinal form. Both forms are becoming more prevalent but the rapid increase of the latter, considered by most authorities to be the more severe, indicates that a greater importance must be accorded to it in the future. The increasing gravity of the situation has accentuated the need for continued research in all aspects of the parasites and their host relationships.

The reason for the increase in prevalence and intensity can be found in the extensive agricultural development which has taken place in the last two decades. In the course of this development the pro-gramme of water conservation has resulted not only in the construction of large numbers of stable artificial reservoirs, but also in more permanent watercourses. The more numerous waterbodies, particularly the large numbers of stable and permanent waterbodies, have led to

greatly increased snail populations, with a consequent increase in the incidence of the infections.

Prior to this programme of intense agricultural development, records show that transmission was at a low level in most areas of the country. This is probably because the natural ecological situations of Rhodesia do not in themselves provide the conditions shown by Shiff (1964a, 1964b) to be necessary for the establishment of dense snail populations. Shiff demonstrated that, of the three species of snails concerned with the transmission of bilharziasis in Rhodesia, only Bulinus (Physopsis) globosus (Morelet) and Bulinus (Physopsis) africanus (Krauss) have the ability to maintain their species in the small temporary waterbodies. He showed that the third species, Biomphalaria pfeifferi (Krauss), is not adapted to survive seasonal desiccation since it has not the ability to reproduce rapidly enough, during short periods of suitable conditions, to re-establish populations decimated by desiccation. Previously this species was probably found only in the few permanent rivers. The numerous permanent waterbodies constructed in the course of the recent water conservation programme have provided conditions well suited to all three species, and the populations are now dense. The records of field surveys by the Bilharziasis Research Laboratory show that there has been a concurrent increase in the prevalence of both forms of the disease.

The extension of this agricultural development programme to the hot low-lying areas in the south and the north of the country, and particularly the development in these areas of irrigation farming with its dense human settlement, can be shown to lead to intense perennial transmission of the infections (McMullen: Restricted Report, 1961). The economic development of Rhodesia demands the establishment of extensive irrigation systems in these areas. When these systems are fully expanded, it is estimated that the production of sugar alone will support nearly one million people who will be concentrated in a small area. If no preventive measures are taken, the disease in these areas must be expected to reach the level of

prevalence and intensity hitherto reported only from Egypt.

There are several possible methods of interrupting the transmission of the parasite. Clarke et al. (1960) and Clarke (1960) have shown that it is possible to reduce the transmission by the control of the snail populations with chemical molluscicides. It has also been shown that snail populations can be reduced by environmental control (Pesigan et al. 1958). However, at present complete eradication of the snails cannot be contemplated, and control measures must be directed to give maximum benefits in the form of prevention of illness and disability. Control measures in the past have been aimed too often solely at reducing the snail populations. Insufficient consideration has been given to reducing the infections in man or reducing the morbidity resulting from these infections. To obtain maximum control of the infections and the morbidity, the existing methods of control must be so employed as to take advantage of any natural processes, for example in the host-parasite relationship, which in themselves help to limit these infections. One such natural process which requires investigation is the possible development of acquired resistance or immunity to infection. The term resistance is used here to describe the ability of a host to resist or withstand an infection by a parasite. Resistance may be innate, or it may be acquired as a result of an infection with the parasite. It may be temporary or permanent, partial or absolute. Resistance is used to describe any ability, whatever its nature, to withstand infection, and it includes immunity. This is a host response to a parasite which may protect the host against further infection, or prevent the development of an infection, or it may inactivate or even cause the death of the parasites of an existing infection. This then is the purpose of the present investigation: to study the effects of acquired resistance or immunity to Schistosoma spp. in man. Little is known of the mechanisms of resistance or immunity in helminthic infections and they may not be the same as those of bacterial or virus infections, which are well understood. However, the purpose

of this study is to investigate the results and effects of resistance on the course of initial or subsequent infections, and no detailed consideration is given to the mechanisms of resistance, the way in which it acts, or the processes by which it is developed.

Bilharziasis has been repeatedly described as essentially a disease of children (Manson-Bahr, 1958; Honey and Gelfand, 1960) and it has been stated that this is because, by the time they have reached adulthood, the people have developed a resistance or an immunity to the infections (Dixon, 1934; Gerber, 1952). It has also been stated that it is a disease of non-indigenous people because such people have had no exposure during childhood by which to develop resistance (Newsome, 1956, quoting Fujinami, 1916). For the hyperendemic irrigation areas of the hot low-lying southern areas of Rhodesia, labour is being recruited largely from the western parts of the country where urinary infections are uncommon and intestinal infections are rare. Many of these people show no indications of previous exposure to infection. It is necessary to study the effects of exposing such people to intense transmission of both forms of the disease. The economically productive adults may well be severely affected by intense infections, with a consequent interference with the economic efficiency of these vital irrigation programmes.

One of the important effects of control programmes, or of the movement of people from non-endemic or hypoendemic areas to hyperendemic areas, would be the development, under certain circumstances, or the destruction under others, of acquired resistance or immunity, if in fact immunity is a feature of the host-parasite relationship in bilharziasis.

The present investigation involves the study of the influence of resistance, if it occurs, on the host-parasite relationship in bilharziasis under different conditions of incidence and transmission of the two species of the genus Schistosoma Weinland, which commonly infect man in Rhodesia. These two species are S. mansoni Sambon and S. capense (Harley). It is usual to refer to the species which

causes the urinary form of the disease in Africa as S. haematobium (Bilharz), but on the authority of le Roux (1958), and because of differences in the snail host and the clinical manifestations, Harley's species has been accepted for the purposes of this study as being distinct from S. haematobium. S. capense will therefore refer to the urinary species of southern Africa.

Studies of the development of acquired resistance in laboratory animals by following an initial immunising infection with exposure to subsequent "challenge" infections give direct evidence of resistance in animals. However, in man it is seldom possible to obtain such direct evidence and the few human experiments must be supported by indirect evidence derived from epidemiological studies. As stated by Vogel (1958), the experimental reinfection of human volunteers is the only completely reliable method of demonstrating the presence or degree of acquired resistance. Until such proof is available, conclusions must be based on epidemiological evidence supporting analogies between the courses of infections in man and in experimental animals.

Geographical conditions which may influence transmission of Schistosoma spp. in Rhodesia.

The term transmission is used here to refer to the passage of parasites from host to host; it may also refer to the degree of the passage of infections. It is reflected in the incidence of infection, which is the number of new infections contracted in a community in a specified period of time.

The level of transmission of Schistosoma spp. is influenced by several geographical factors, including

(a) the density of the human population (Pitchford, 1958);

(b) the density of snail populations, which is influenced by temperature, rainfall and permanence and size of waterbodies (Shiff, 1964a, 1964b).

Rhodesia occupies an area of south central Africa of 389,000 square kilometers lying between the Zambesi and Limpopo rivers (15° to 22°S. latitude and 26° to 34°E. longitude). There is a broad ridge running across the country from the south-west to the north-east which forms the central plateau and watershed of the major rivers. From this ridge the river systems drain to the north into the Zambesi river, and to the south into the Sabi and Limpopo rivers.

The European population of Rhodesia is at present 217,000 and there are 3,900,000 Africans. The population is concentrated on the central plateau. The hot low-lying areas to the south and north of the country remained sparsely populated and totally undeveloped until the control of malaria in recent years allowed the settlement of these areas. The population of the low altitude areas is now increasing, particularly in well defined isolated communities such as irrigation settlements.

The climatic conditions of the central plateau of Rhodesia permit the definition of four distinct seasons. The main rainfall occurs during the summer in a short season lasting from December to February. From March to May there is a period of little rain, with moderate to high temperatures. This is followed by the winter lasting from June to August, when virtually no rain falls, and when temperatures vary diurnally from below freezing point to as high as 20°C. Lastly, from September to mid-December is the hot, dry season, relieved during November and December by occasional heavy showers of rain. The mean annual rainfall of the central plateau varies from over 90 cm. in the north-east, to below 30 cm. in the south-west.

The low altitude areas have similar seasons, but the rainfall is less, and the seasonal temperature variations are less marked. The temperatures are considerably higher. The mean annual rainfall is usually below 25 cm., and in some areas it may be below 15 cm.

PART I.

METHODS AND TECHNIQUES.

The methods and techniques employed in the course of this study have been followed with rigid attention to detail and, because small variations in technique would be reflected in the results, full details of the methods and techniques are given.

1. URINE EXAMINATION.

A. Collection of Specimens.

Bennie (1949) and Jordan (1963) have both reported on the diurnal variations in the output of S. haematobium eggs in urines from infected individuals. Bennie used a sedimentation technique by which she obtained an estimate of the number of eggs in the whole urine specimen; Jordan counted the eggs present in 10ml. of the urine. Both demonstrated that maximum egg release took place at the middle of the day. Jordan showed that there was a regular increase in egg release from relatively small numbers in the early morning to a peak occurring between noon and 2 p.m. For practical reasons it was not possible for urine samples to be collected at the period of peak egg output, and it was necessary to limit the time of collecting of specimens to the period between 9 a.m. and 2 p.m.

In all the cases the name, sex, age, length of residence in the area, and the history of previous treatment for bilharziasis were recorded for each person examined. Great difficulty was experienced in assessing the ages, particularly of the children, because it is rare for an African to be sure of his age. In an attempt to ensure a minimum of error, consideration was given to the following points in the assessment of the age of each individual:-

(a) the individual was questioned regarding age;

(b) an independent assessment of age was made by the investigator;

(c) an intelligent, educated member of the community,

usually a school-teacher, was asked to give information on the individual;

(d) in some cases the individuals were questioned on their memories of abnormal seasons or important events. In many cases, questioning of parents in this way gave accurate estimation of ages of children. From all this available information, an assessment of the age of each individual was made.

Throughout all the surveys, results of the examination of persons having had treatment for the disease, within a period of three months prior to the survey, were disregarded in the analysis of results.

A commonly occurring fault in reported urine examination surveys has been the lack of standardisation of the methods of taking samples. Most reports refer to the number of eggs in 10ml. of urine, without reference to the total volume of urine or the time the urine was retained in the bladder. Since egg counting should attempt to measure the rate at which eggs are passing through the bladder, it appears more logical to relate the number of eggs counted to the time they have taken to pass into the bladder, rather than to a standard volume of urine. This argument becomes more acceptable when it is realised that marked variations in renal activity occur in individuals, the results of surveys showing that the volume of urine passed in one hour varies from below 10ml. to over 100ml. For this reason, in the majority of surveys undertaken in the course of the present study, the total egg output was estimated from a urine sample representing exactly one hour of urine collection in the bladder, and therefore to one hour of egg passage into the bladder.

The subjects to be examined were first made to empty their bladders into large, wide-mouthed, screw-capped bottles. These bottles were collected as evidence that the bladders had been emptied. After exactly one hour a second specimen was collected in a 200ml., wide-mouthed, screw-capped bottle. To facilitate the organisation and

management of surveys covering large numbers of people, they were arranged in groups of twenty, each with a colour code. In this way it was found possible to ensure that the second specimens were passed without delay at the end of the hour period.

When these urine samples were collected they were immediately preserved by the addition of 1ml. of an aqueous solution of merthiolate and sodium citrate. This quantity of solution contained sufficient of the chemicals to give final concentrations of not less than 0.01% and 0.1% respectively. Each urine specimen was measured for volume, allowance being made for the 1ml. of preservative solution. The preserved specimens were then returned to the laboratory for examination.

Two routine procedures were used for the examination of the urine specimens for eggs and for the counting of the eggs; these were the direct sediment examination method, and a modification of the method of Bell and Bradley (Bell: personal communication).

B. Direct Sediment Examination Method.

The whole specimen was allowed to stand in its bottle for 30 minutes to allow the sediment, including the eggs, to be deposited; it was then decanted slowly and steadily to prevent the disturbance of the sediment, to leave 12 to 14ml. of urine containing the sediment in the bottle. The bottle was vigorously shaken and the contents transferred to a 15ml. conical centrifuge tube and centrifuged for one minute at approximately 1,500 r.p.m. The supernatant urine in the centrifuge tube was carefully decanted to leave 0.5ml. of the urine, with the sediment, in the tube. This particular operation proved difficult and it was impossible to ensure that exactly 0.5ml. was left since expense precluded the use of graduated tubes for surveys of this magnitude. The tube was then flicked several times with the fingers to bring the sediment into suspension, and 0.05ml. of the suspension was removed with a pipette for microscopic examination.

Where eggs were present they were counted. If no eggs were found the tube was again agitated and a large drop was poured onto a slide for further examination. The number of eggs on the slide was accep-

ted as representing one tenth of the total number of eggs present in the whole specimen of the urine. If no eggs were found in the initial measured 0.05ml. sample but were present in the second examination, no count was made, the result being recorded simply as 0. This was necessary for statistical analysis of results.

This method of sediment examination, although satisfactory for prevalence surveys, was not fully satisfactory for estimating egg production because of errors inherent in the technique.

C. Modified Method of Bell and Bradley (Bell: personal communication.)

This technique gave the most consistent results when egg counts from urine samples were required. The apparatus designed and made for the purpose is pictured in Fig. 1.

The apparatus consists of a sintered glass filter disc 3 cm. in diameter, inlaid in a plastic base plate, leading to a powerful suction pump. Above the plastic plate is a 10ml. graduated pipette attached through a two-way valve to a pipette filler bulb on the one side, and a reservoir of strong detergent solution on the other. A rubber bulb pressure pump is used to force the detergent through the system when required. A brass cylinder, 7 cm. in length with an internal diameter of 3 cm., completes the apparatus: this cylinder is heavy, weighing 450 gm. to ensure the tight seal between it and the filter paper required to prevent leakage.

The preserved urine sample is thoroughly shaken to obtain an even distribution of any eggs present, and a 10ml. aliquot is drawn up into the pipette, using the pipette filler bulb. A 5 cm. square of Whatman No. 1 chromatographic grade filter paper is placed over the sintered glass disc and moistened with water to ensure contact with the plastic base. The brass cylinder is placed on the filter paper directly over the filter disc, the correct position being located by guide blocks. The suction pump is operated and the urine sample is run into the brass cylinder from the pipette. The powerful suction usually draws the fluid through the filter paper in a matter of seconds, leaving

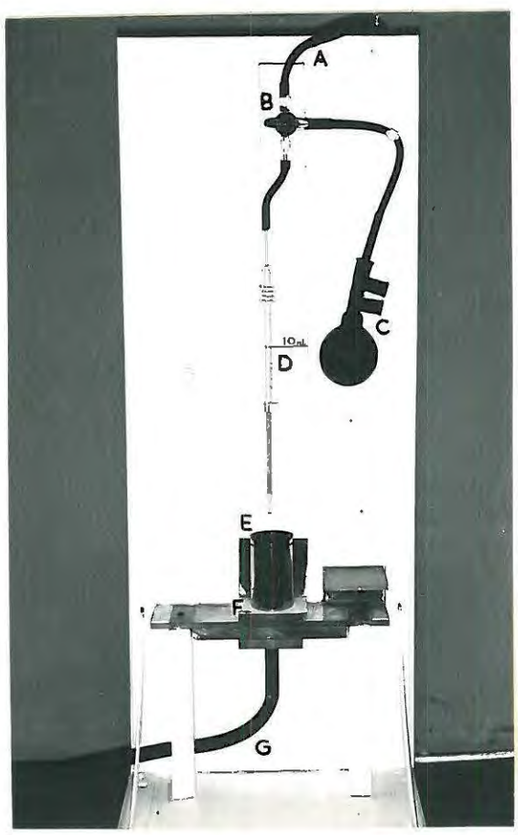


Figure 1. Modified Bell-Bradley apparatus (Bell: personal communication) for recovering eggs of Schistosoma spp. from urine.

A - tube from reservoir of detergent solution
B - glass two-way valve
C - rubber pipette filler
D - graduated pipette
E - brass cylinder
F - filter paper on sintered glass filter disc
G - tube from filter to suction apparatus.

the eggs on the paper. The direction of the two-way valve is then reversed and the pipette is flushed under pressure with detergent. The end of the pipette and the brass cylinder are then washed with a strong jet from a wash bottle. When all the washing fluid has been drawn through the paper, the latter is removed and floated onto a large drop of a saturated aqueous solution of ninhydrin on a glass plate, then dried and developed for colour in a hot-air oven at 60°C. for one hour.

The filter paper is examined through a standard microscope at a magnification of 40x. The eggs are distinct, having a dark blue-black colour (Fig. 2) and it is simple to distinguish between the eggs of S. capense and S. mansoni.

2. STOOL EXAMINATION.

The collection of stool specimens for surveys follows a similar pattern to the collection of urine specimens and the name, sex, age, length of residence in the area, and the history of previous treatment for bilharziasis was recorded for each person examined. Waxed paper cartons were issued to each person and they were asked to produce specimens on the same day. After the cartons were returned approximately 10 gm. of each faecal sample was transferred to a 200ml. wide-mouthed, screw-capped bottle, and emulsified in approximately 30 ml. of 10% formalin in 1% aqueous saline. The emulsified stool sample was returned to the central laboratory for examination.

In the next stage in the preparation of the sample for examination, the emulsified specimen was diluted with a large volume of 1% saline solution and strained through a fine copper gauze having a pore size of 0.15 square mm. The strained suspension was repeatedly washed in saline by allowing the sediment to settle and then decanting the liquid. After the final washing the supernatant fluid was reduced to 10 to 15ml., including the sediment; this was transferred to a 15ml. conical centrifuge tube, shaken with 0.5ml. of ether and centrifuged for one minute at approximately 1,500 r.p.m.

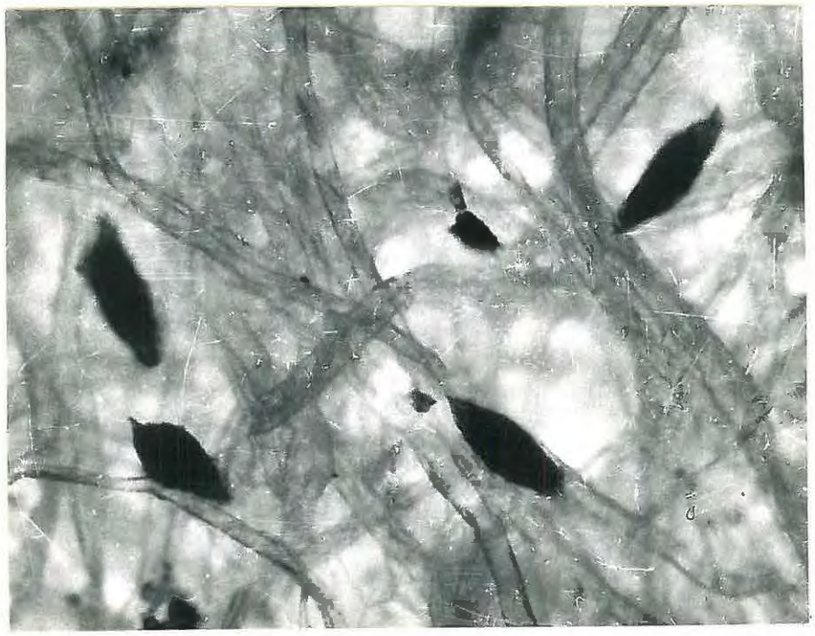


Figure 2. S. capense eggs trapped on filter paper by modified Bell and Bradley apparatus. Stained with saturated aqueous ninhydrin.

The plug of fat left by the evaporated ether was removed with a needle and the tube decanted to leave approximately 0.5ml. of the liquid and sediment. After violent agitation, a drop of the suspension was transferred to a slide and examined for eggs. It was not found necessary to stain the eggs.

3. FLUORESCENT ANTIBODY TEST SURVEYS.

The methods used were modified from the indirect fluorescent antibody techniques of Sadun et al. (1960, 1961a), Anderson et al. (1961a, 1961b), and Cookson (1963).

A. Collection and Processing of Blood Samples.

The name, sex, age, length of residence in the area, and the history of previous treatment for bilharziasis were recorded for each person to be tested for antibodies. The third finger was cleaned with a swab soaked in alcohol and, using a No. 6D triangular section surgical suture needle, the finger was punctured and several drops of blood were squeezed out onto a strip of Whatman No. 1 filter paper. Sufficient blood was collected to saturate an area of filter paper of approximately 5 square cm.; it was allowed to dry, and the sample could be stored in the dry state for several months if necessary.

The blot of dried blood was carefully cut out, rolled into a cylinder and introduced into the bore of a 6 cm. length of tygon tube of 4 mm. internal diameter, one end of which was blocked with a steel ball-bearing of 5 mm. diameter. 0.1ml. phosphate buffered 0.9% saline, pH 7.1, 0.01 M., was introduced into the tube using a syringe fitted with a 22 gauge needle. The tube was then rolled between the thumb and fore-finger to ensure thorough wetting of the paper and dried blood, and allowed to stand for one hour. At the end of this period the tube was again rolled between the thumb and fore-finger and then, using a very small engineer's vice, the eluate was squeezed out of the tygon tubing into a small glass centrifuge tube (7 cm. long, 5 mm. internal diameter) containing the cercariae to be used as the source of antigen.

B. Collection and Preservation of Cercariae for use as Antigen Source.

The cercariae were obtained from laboratory-bred Biomphalaria pfeifferi and Bulinus (Physopsis) globosus exposed previously to three miracidia each of S. mansoni and S. capense respectively. The infected snails were placed in tubes each containing a small quantity of water, and allowed to stand for one to two hours under strong light to stimulate the release of cercariae. After this period, the water from each tube was strained through surgical gauze to remove debris and snail faeces, and then pooled. Buffered formalin solution was added to give a final concentration of 5% formalin in a phosphate buffered solution of pH 7.1. The container was allowed to stand for one hour to allow the now dead cercariae to sink to the bottom, and the supernatant liquid was decanted. The cercariae were washed in buffered formalin and the final volume adjusted so that 0.5ml. of the buffered formalin contained approximately 100 cercariae. To this suspension was added sufficient commercial counter stain (Difco F.T.A. counter stain), consisting of bovine albumin conjugated to lissamine rhodamine RB.200, to give a final concentration of 1 : 200. This resulted in the coating of the cercariae with the rhodamine conjugated bovine albumin which prevented mechanical absorption of the fluorescent Coomb's serum used in the indirect fluorescent antibody test. The cercarial suspension was stored at 4°C. for a maximum of four weeks in phosphate buffered saline pH 7.1, 0.01 M., containing 5% formalin.

C. Preparation of Fluorescein labelled Coomb's Serum.

Crude Coomb's serum (goat, rabbit or horse anti-human serum), purchased commercially as a lyophilised powder, was reconstituted in distilled water to its original volume, then diluted to three times its original volume in sodium carbonate-sodium bicarbonate buffer, pH 9.0, 0.5 M. Fluorescein iso-thiocyanate was dissolved in the minimum quantity of acetone from which all traces of water had been

removed. This acetone solution was added drop by drop to the buffered Coomb's serum solution which was stirred as vigorously as possible without frothing, at 4°C. Sufficient fluorescein solution was added to allow 3 mg. of fluorescein iso-thiocyanate for each millilitre of original Coomb's serum.

Conjugation was allowed to take place over a period of 24 hours, the solution being stirred constantly and kept at 4°C. for the whole period. After conjugation, the globulin fraction was collected by chromatographic separation through a sephadex column. The pH of the globulin solution was adjusted by dialysis in phosphate buffered saline solution (P.B.S.) pH 7.1, 0.01 M.

The reactivity of the fluorescent anti-human globulin, which could be stored for long periods at -4°C., was assessed by titre estimations against known schistosome anti-sera, using the indirect technique.

D. The Fluorescent Antibody Reaction.

In the indirect fluorescent antibody technique the known antigen, the cercariae, was reacted with the test human serum - in this case the eluate from the blood collected on filter paper. The presence of the antigen-antibody complex was demonstrated using the fluorescent anti-human globulin.

Prior to use, the cercariae were repeatedly washed in P.B.S. by centrifugation, removal of the supernatant, and resuspension in fresh P.B.S. After the final washing as much of the fluid as possible was removed to leave a small knot of cercariae in the bottom of the tube. The eluate from the blood-soaked filter paper (as much as 0.06ml. can be recovered) was run into the tube and the cercariae and the test serum eluate were gently agitated together for 30 minutes at room temperature. The cercariae were again washed three times in P.B.S. and, after the final washing, as much fluid as possible was removed. 0.05ml. of the fluorescein conjugated anti-human globulin solution was run into the tube and agitated with the cercariae for 15 minutes. Again the cercariae were washed three times in P.B.S.

and drained of excess fluid, and two to three drops of 10% glycerine in P.B.S. were added to prevent drying out of the cercariae.

The cercariae were transferred to a slide and examined at 50x magnification through a microscope fitted with a source of ultra-violet light. Filters were used to give UV or UV-blue light and an ultra-violet exclusion filter cap was fitted over the eye-piece of the microscope.

Negative tests where no schistosome antibody was present in the test serum showed only the brick-red colour of the rhodamine on the cercariae; in positive tests, with serum containing the antibody, the rhodamine colour of the cercariae was overlaid by the very strong yellow-green fluorescence of the fluorescein labelled Coomb's serum. The degree of overlay of the fluorescein over the rhodamine varied and a test was read as positive if the periphery of the image of the cercaria showed a bright and distinct fluorescein fluorescence (Figs.3 and 4).

4. INTRADERMAL TESTS.

Antigen for use in intradermal tests was prepared from adult worms by the methods of Melcher (1943) from worms recovered from infected mice by perfusion of the mesenteric veins, the portal vein and the liver. As well as this, antigen prepared by the same method was obtained from the World Health Organisation.

The name, sex, age, length of residence in the area, and the history of previous treatment for bilharziasis were recorded for each person examined. The scapular region of the back was cleaned with a swab soaked in alcohol and, using two 1ml. tuberculin syringes fitted with 27 gauge, 1 cm. long platinum needles which could be flame sterilised, 0.05ml. of each of the antigen and a control solution were injected intradermally, about 6 cm. apart in the cleaned area. The result was read after 15 minutes and the test was regarded as positive if the antigen wheal had increased in size to twice the diameter of the control wheal, or to over 1 square cm. in area (Fig. 5). A permanent record of the result of the test can be made

Figure 3. Cercaria used for the fluorescent antibody test. Brick red coloud indicates that no antigen-antibody reaction has taken place.

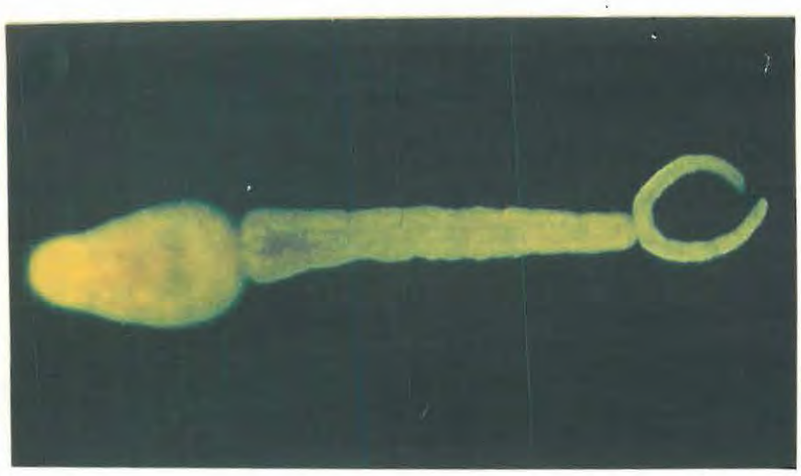


Figure 4. Cercaria used for the fluorescent antibody test. Yellow green colour superimposed on the original brick red indicates that antibodies were present.

by outlining the wheals with a ball-point pen and pressing an alcohol-moistened piece of paper against the marks, the ball-point outline being transferred to the paper (Fig. 6).

5. EXPOSURE OF MICE TO INFECTION.

The mice used in experimental infections were exposed to the cercariae by one of two methods, the first by subcutaneous introduction of the cercariae, and the second by percutaneous exposure.

A. Subcutaneous Exposure to Infection.

Cercariae were collected from laboratory-bred snails exposed originally to three miracidia each. The snails were placed in small quantities of water in tubes and exposed to strong light for an hour to stimulate the release of cercariae. After this period the water samples with the cercariae from a minimum of ten snails were pooled after straining through a fine nylon mesh of surgical gauze to remove solid debris and snail faeces. The number of cercariae in a unit volume of water was estimated by counting the numbers in small aliquot samples.

Each mouse was held by the skin of the dorsal surface of the neck and by the tail, and the required quantity of cercarial suspension was introduced subcutaneously in the dorsal thoracic region using a 2ml. hypodermic syringe fitted with a 20 gauge, 3 cm. needle. Care was taken to ensure that the cercariae were not more than four hours old when used.

B. Percutaneous Exposure to Infection.

Cercariae were collected and counted as for the method of subcutaneous infection.

Prior to exposure the mice were made to paddle in water approximately 1 cm. deep for ten minutes to stimulate defaecation and urination. The required number of cercariae were added to sufficient water in a separate container, usually 10 to 12 cm. in diameter, to give a depth of water containing the cercariae of 1 to

Figure 5. Test wheal (left) and control wheal (right) 15 minutes after intradermal injection of antigen and control in a person with chronic bilharziasis.

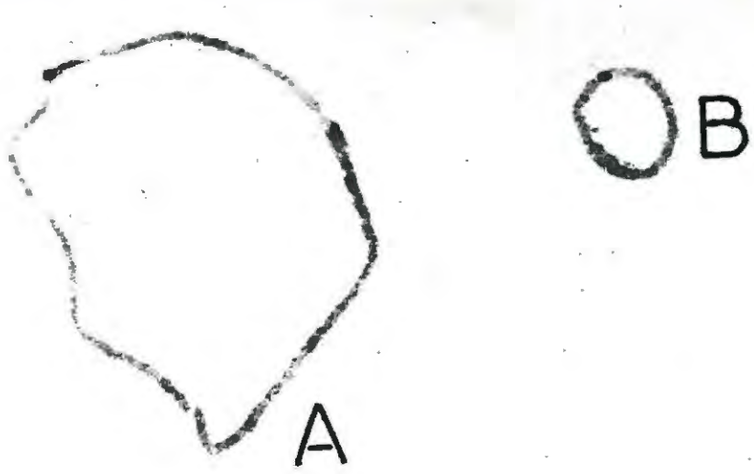


Figure 6. Transfer of inked outline of wheals to alcohol moistened paper. Test wheal (A) and control wheal (B). from same test as illustrated in fig. 5.

2 cm. Each mouse was exposed individually by making it paddle in the infected water for one hour before being transferred back to the cages.

6. PERFUSION OF MICE TO RECOVER WORMS.

The procedure used was modified from the methods of Yolles et al. (1947). The mice were killed by intra-peritoneal injection of an excess of nembutal which not only killed the mice but also narcotised the worms in the mesenteric veins, causing them to be swept back to the portal vein or the liver. The animal was skinned by tearing the skin from the umbilical area and stripping it off from the whole thoracic and abdominal region with a single pull. The abdominal wall was also removed by tearing it with a single pull. Not only did the tearing off of the skin and abdominal wall save considerable time but it also resulted in less blood loss than encountered when the animal was carefully dissected. The rib cage was cut away with scissors, care being taken to prevent cutting any large artery or vein. The animal was held over a large conical flask and the hepatic portal vein was cut. A pulsating thrust of isotonic saline was introduced through a 20 gauge, 3 cm. syringe needle into the inferior vena cava, with the needle point in the direction of the liver and almost in the hepatic sinus. The pulsating thrust of saline was delivered at approximately 20 thrusts a minute at a pressure between 0.6 and 0.8 Kg./square cm. After 10 to 15 seconds of perfusion of the liver the needle was transferred to the thoracic aorta where it was slid into the aorta for approximately 1 cm. towards the abdomen, with the point reaching the position of the diaphragm. Again perfusion was maintained for 15 seconds before the needle was removed and the animal washed in saline solution to free any worms wedged in the peritoneal cavity. 4% sodium citrate solution in water was added to the perfusate in the jar to prevent the formation of blood clots and a few drops of 1% saponin solution were added to induce haemolysis. The worms were recovered from the sludge that settled to

the bottom of the jar. After perfusion, the mesenteric veins were examined for the presence of any remaining worms but it was seldom that any were found. The liver was also removed, crushed and examined for worms.

PART II.

THE DEVELOPMENT IN ANIMALS OF ACQUIRED RESISTANCE TO INFECTIONS OF SCHISTOSOMA SPP.

It is not possible to draw direct analogies on the changes in the course of infections of Schistosoma spp., and on the host parasite relationships, between experimental animals and man. However, if such analogies can be supported by direct or indirect evidence of similar changes in man, then they are more justifiable. Consequently, there has been considerable interest in the demonstration in laboratory animals of acquired resistance to schistosome infections. Acquired resistance is the protection against infection which is developed in an animal as a result of a previous infection, or as a result of artificial immunisation.

Many animals have been used to demonstrate developed protection against schistosome infections. The most commonly used laboratory host animal is the rhesus monkey Macaca mulatta. This monkey has been used to demonstrate acquired resistance to S. mansoni and S. japonicum infections. Its use has both advantages and disadvantages. M. mulatta has a strong natural resistance to infection by both these species of parasite, and infections die out within four years. However, it can be used for the demonstration of acquired resistance since this develops rapidly. Since the animal is refractory to the infections, any demonstrated resistance or immunity must be considered to be both innate and acquired. Monkeys of the genus Cercopithecus have also been used for experimental purposes. This monkey is also unsuitable since it is highly susceptible to infection, and the infections are persistent; little resistance can be demonstrated. The rabbit Oryctolagus cuniculus, the white rat Rattus rattus, the golden hamster Mesocricetus auretus, and the white mouse Mus musculus, have also been widely used. Most experiments to investigate acquired resistance in animals have involved the use of the parasite species S. mansoni or S. japonicum, but some workers have used the allied

parasite Schistosomatium douthitti. No controlled experimental immunological studies using either S. capense or S. haematobium have been reported.

None of the reported investigations on acquired resistance can be accepted as dealing with a parasite in its natural definitive host, and the results may therefore reflect not only acquired resistance but also the additive effects of innate resistance. In some cases, however, the acquisition of resistance may be hindered by high susceptibility. In all such experiments, therefore, the results can only be accepted as applying to the particular host species used, and not to any other.

In the majority of the reports, resistance has been demonstrated by following an initial infection or series of infections, referred to as the immunising infections, with a heavy later infection, referred to as the challenge infection. The results are compared with those from control animals which have received the equivalent of the challenge infection without the immunising infection. The resistance may be manifested in many ways. The number of eggs passed by the animal may be lower than in the controls, the number of worms maturing from the challenge infections may be decreased, and these worms may be stunted. In addition, the pathological changes resulting from the infection may be lessened, and the animals may be able to survive challenge infections which would be lethal to non-immune animals. The immunising effects of infection with attenuated but still living parasites, achieved by exposing the animals to irradiated cercariae have been studied. There have been reports on the effects of antigenic stimulation with antigens derived from dead worm material (somatic antigens) and from secretory or excretory material (metabolic antigens). The demonstration of passive transfer of resistance by the transfer of serum from immune to non-immune experimental animals has also been attempted.

Challenge infections have been most often in the form of superinfection where the animal has been exposed to infection additional to the still living immunising infection. However, the effects of

re-infection, where the animal is exposed to challenge infection after the death of the original immunising infection, have also been reported. This difference is important since if the protection persists after the death of the worms of the immunising infection, and after the removal or decay of any antigenic material, e.g. eggs, it indicates a true immune response which is independent of the presence of antigen.

The decrease in the numbers of worms maturing from challenge infections in immunised animals, compared with normal animals subjected to the same infections, was first reported in detail by Vogel and Minning (1953), who studied the course of infections of S. japonicum in rhesus monkeys. They reported almost complete protection conferred on the monkeys after periods of up to 30 months, with few of the challenge cercariae maturing into adult worms. The monkeys were able to withstand without ill-effect repeated challenge exposures to double the number of cercariae which would have been lethal to non-immune monkeys. They also reported that some of the monkeys were treated with drugs for the immunising infections after periods of up to 600 days, and then challenged to normally lethal numbers of cercariae. These animals also survived the infections and, when subsequently sacrificed, no worms were recovered and nc eggs were found in the liver. In spite of the use of rhesus monkeys for these experiments, the results are valid since adequate non-immunised control monkeys were also used. However, there can be nc proof that, after the chemotherapeutic treatment, there was no antigenic material from the dead worms remaining in the monkeys at the time of the challenge.

Kagan and Lee (1953) found that, in mice, resistance to S. douthitti was destroyed by chemotherapeutic treatment. However, they had allowed a maximum immunising period of three months between the initial infection and the drug treatment, and this is possibly too short a time to allow for the development of a permanent immunity. Vogel and Minning (op. cit.) working on a longer immunisation period, found that almost perfect protection was possible even after using only male cercariae for the immunising infection, and after drug

treatment the challenge reinfection resulted in the recovery of only very few eggs. The available data, therefore, indicates that rhesus monkeys are able to develop a permanent immunity which persists after the removal of living worms, and probably of antigenic material. The fact that the work of Vogel and Minning was undertaken in rhesus monkeys detracts slightly from the value of the results.

Kagan (1952) reported a stunting of S. douthitti worms in challenge infections in previously immunised mice even after single sex immunisation. He found that female worms gave rise to more complete resistance than a male worm infection, but that the strongest resistance, as demonstrated by the stunting of surviving worms, resulted from bisexual infections. Meleney and Moore (1954) found little evidence of resistance to S. mansoni in rhesus monkeys following a single sex immunising infection. However, they had allowed for an immunising period of only 11 to 12 weeks which is possibly too short a time for the development of resistance under these circumstances. Smithers (1962a) reported that resistance to male worm infections took longer to develop than the resistance to infections of both sexes.

Olivier and Schneidermann (1953) studied the course of S. mansoni infections in white mice and hamsters and they reported decreased worm loads in immunised animals following heavy challenge exposure, and also that what was presumed to be an acquired resistance delayed the death of the immunised mice after these heavy challenge exposures.

Thompson (1954), working with S. mansoni in mice, reported some evidence of acquired resistance following repeated light infections, but little resistance resulting from single original infections. He suggested that man's ability to survive in endemic areas was more the result of a natural resistance than of acquired resistance, and he questioned the strong resistance reported by other authors. Kagan (1958), working with S. douthitti infections in mice, also reported that repeated light exposure to infections of both sexes increased the resistance. Lurie and de Meillon (1957) reported that, in mice exposed in this manner to infections of S. mansoni,

only 1.5 percent of the cercariae developed into adult worms compared with a recovery of 23.5 percent from a single exposure to approximately the same total number of cercariae. They also showed that the pathology of the disease followed a different course, developing more slowly after repeated infections than after a single exposure. However, after a period of 24 weeks the extent of pathological change was the same for both types of exposure. They found that the infections resulting from a single exposure showed a tendency to spontaneous cure after 40 to 48 weeks. No great importance was attached to this finding although it probably represents the development of the strongest resistance.

Newsome (1956) immunised baboons (Papio hamadryas) with S. mansoni and he found that they could subsequently withstand many more cercariae than the number which normally proves lethal for this species of host. His animals were eventually able to withstand challenge exposures of over 100,000 cercariae without marked ill-effect. This represents a colossal infection and the ability of the animals to withstand this infection demonstrates a very strong protection. Vogel (1958) also reported on the ability of animals to survive normally lethal exposures to infection. Working with S. japonicum infections in rhesus monkeys he found that the level of lethal exposure was between 400 and 600 cercariae. When he attempted to immunise an animal with 200 cercariae and then challenged after nine months with just over 600 cercariae, the animal died whereas if he immunised the animal with 250 cercariae and then challenged after a year, again with 600 cercariae, eggs were passed but the animal survived the infection.

The significance of these results lies in the demonstration that the numbers of cercariae used in the immunising infections, and the length of time the immunisation is allowed to act, are critical factors in the development of resistance. A subsequent re-challenge with 1,200 cercariae 18 months after the immunising infection resulted in very few eggs, and no deaths took place. von Lichtenberg and Ritchie (1961) studied S. mansoni infections in rhesus

monkeys and they found that the resistant animals were able to survive two challenge exposures, each to well over double the numbers of cercariae normally lethal to the monkeys. In a carefully controlled series of experiments they observed that almost complete protection against subsequent super-infections was developed in the monkeys. They reported a decrease in the numbers of worms maturing from the challenge infections from 51, 14 and 29 percent in non-resistant control animals to 10, 9, 10 and 0.2 percent in four test animals sacrificed after the challenge infections. They observed that the few worms which did reach maturity were stunted in size. They also studied the cellular reactions to the invading parasites of the two challenge exposures and they claimed to show a true cellular resistance in the resistant animals. The schistosomulae tended to die in three sites, in the skin, in the lungs and in the portal system, with greatly reduced histo-pathology in the host, although there was a temporary but severe local reaction round the invading schistosomulae. Of the few schistosomulae that did reach the portal system, most were stunted and short lived. Their claim that this demonstrated true cellular resistance must be accepted.

Davis et al. (1963) compared the results of exposure of resistant and non-resistant rhesus monkeys to 10,000 cercariae of S. mansoni. They demonstrated a more marked skin reaction in immune monkeys even when the site of the challenge infection was separated from the site of the previous immunising infection. Many of the cercariae were found to have died in the skin, and fewer worms were recovered from the animals when they were sacrificed.

Ritchie et al (1963) immunised mice, rats and hamsters with S. mansoni and then subsequently challenged with the same species. With an immunising period of 24 weeks between the initial and challenge infections there was a marked decrease in the number of worms recovered at autopsy, illustrating the development of strong resistance. However, when a longer time was allowed to elapse between the two infections, the degree of resistance appeared to

decrease.

The examination of the pattern of egg release by infected animals furnishes additional evidence of the development of resistance. Cram and Files (1947), Standen (1949), Meleney and Moore (1954) and von Lichtenberg and Ritchie (1961) all reported on the decrease in egg release from rhesus monkeys infected with S. mansoni after an initial period of high egg production. They also demonstrated that there was little or no subsequent increase in the egg release after challenge infections. Vogel and Minning (1953) reported that when rhesus monkeys were exposed to infection by S. japonicum, egg production rose to a peak in the early months of the infection, then production decreased and then stopped altogether. Challenge infections after the period of peak egg production raised the production for a further short period, but never to the level of the first peak. Vogel (1958) confirmed this finding even when the challenge infection was at an abnormally high level. Hsü and Hsü (1961a) observed marked differences between animals immunised with the Formosan strain of S. japonicum and non-resistant animals when both groups were challenged with the Japanese strain of the same species. The immunised animals produced fewer eggs and they survived longer than the non-resistant animals. This is particularly interesting since the Formosan strain of S. japonicum does not mature in either man or rhesus monkeys and therefore infection with this strain is similar to the use of attenuated cercariae.

Ritchie et al. (1964) studied the egg production in Cercopithecus sp. infected with S. mansoni. The monkeys were the St. Kitts green monkeys - no species name was given. Only a slight decrease in egg production was observed in this monkey which retains its infection much longer than the rhesus monkey.

Naimark et al. (1960) conducted a detailed examination of S. mansoni infections in rhesus monkeys. Resistance was demonstrated by a decrease in egg production after the peak of production and histological examination of the monkeys at autopsy showed that this was the result of death of the worms. The decrease in egg production could

not have been due to death of the worms or to fibrotic obstruction of egg passage through the bowel wall. They demonstrated the variations in response by individual hosts and the influence of the initial exposure patterns on the subsequent development of resistance.

The results of Naimark and his co-workers were confirmed by Jachowski et al. (1963) who also studied S. mansoni infections in rhesus monkeys. Since it is ethically impossible to conduct such studies in man, but since it is apparent that similar trends in egg production take place in man, this work on monkeys is discussed in detail. This work was not designed to show the patterns of egg release but the authors recorded egg production from monkeys which had single, double or repeated exposure to infection, and the results are essentially similar to those of other workers.

Jackowski and his co-authors exposed rhesus monkeys to three different exposure patterns. Group A, comprising four monkeys, was exposed to a single infection of 1,000 cercariae; group B, made up of two monkeys, was exposed to two infections of 500 cercariae with a seven month interval between exposures; group C, comprising two monkeys, received multiple exposures of between 25 and 50 cercariae for each exposure at 35 day intervals. The faeces of all these animals were examined at 50 day intervals for the first 23 months after the initial infections, and at 100 day intervals for the next 17 months. Estimates were made of the numbers of S. mansoni eggs per gram of faeces. These results are given in Table 1 and Figure 7.

The results illustrate that the S. mansoni egg production rises to a peak and then, slowly or rapidly, decreases and this pattern is influenced by the worm burden and the pattern of exposure. Single heavy exposure resulted in a very rapid rise in egg production which reached a peak approximately three to four months after infection. Thereafter, egg production decreased rapidly to low numbers after six months and production virtually ceased after 21 months of infection.

Where two exposures were given, with an interval of seven months between the exposures, egg production remained low until after the

TABLE 1.

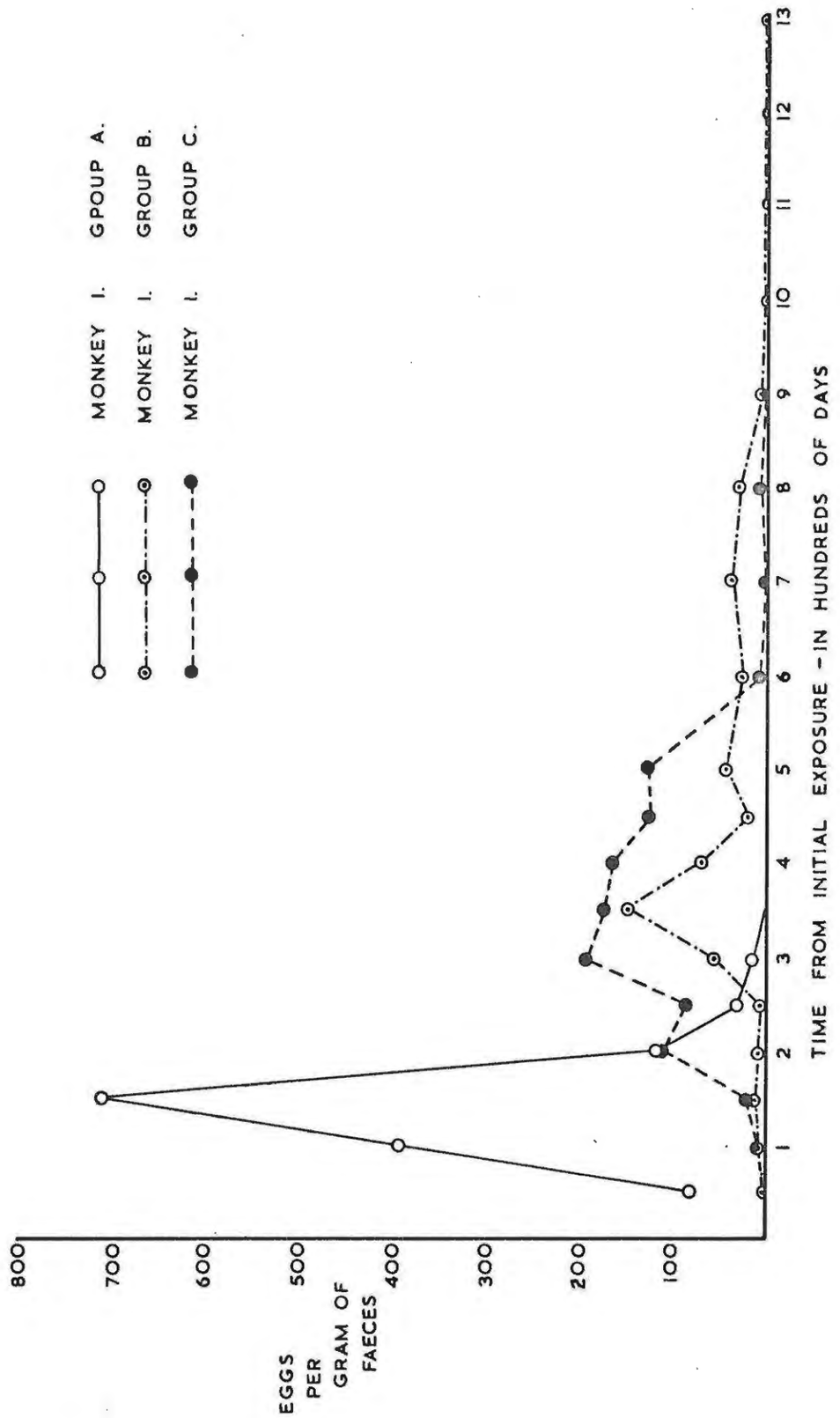
From Jachowski et al. (1963) : Egg production from three groups of M. mulatta monkeys exposed to different patterns of infection of S. mansoni.

Group A monkeys received a single immunising infection of 1000 cercariae and they were challenged to 500 and then 5000 cercariae at different times after 700 days.
Group B monkeys received two immunising infections at 250-day interval.
Group C monkeys received repeated exposures of between 25 and 50 cercariae every 35 days.

	Eggs per gram of faeces																		
Group A monkey 1	0	76	395	712	117	32	16	0+	0+	0	0+	0	0	0	0	0	0	0	
2	0	57	279	88	22	1	1	0+	0+	1	1	0+	0	0+	1	0	0	0	
3	0	28	342	207	18	4	7	4	3	0+	0+	0+	0	0+	0+	1	0+	2	
4	0	89	324	55	27	5	3	0+	0										
5	0	0	0	0	0	0	0	0	0										
Group B monkey 1	0	2	6	12	8	3	54	147	70	18	46	24	38	31	4	0+	2	0+	0+
2	0	2	0+	0+	0+	0+	8	35	55	2	25	13	20	13	4	2	15	3	1
Group C monkey 1	0	0	8	23	112	85	193	174	164	128	127	8	0+	5					
2	0	0	7	4	35	133	185	313	490	222	74	7	7	7					
			1		2		3		4		5	6	7	8	9	10	11	12	13
	Time, in hundreds of days.																		

0+ = Mean count less than 1 egg per gram of faeces.

Figure 7. Production of S. mansoni eggs by rhesus monkeys exposed to different patterns of infection. (From the data of Jachowski et al., 1963)



second exposure, after which there was a rise to a peak ten months after the first exposure. The subsequent fall in egg production was not rapid and, although by the 30th month the egg production was low, the monkeys never ceased passing eggs.

Where multiple exposures were given, egg production increased slowly to a high peak 13 months after the initial infection and it then dropped slowly to a low level without actually ceasing. The peak was reached after exposure to only 500 to 600 cercariae which was only just over half the number given to groups A and B.

When comparing groups A and B it is clear that the decrease in egg production in group A was not the result of natural senescence of the worms, since in group B egg production continued for a much longer time, even after the second exposure. The decrease must then have been the result of some change in the host parasite relationship. Whether this was the result of antibody formation or not, there was a protection conferred on the hosts. Group A was subsequently challenged with 5,000 cercariae, normally sufficient to kill monkeys of this species, 22 months after exposure, and again at a later date. There was no increase in egg production.

It appears that the strongest resistance was acquired after a single heavy exposure. In the series with low level multiple exposures, the final peak of egg production was reached after a longer period and resistance developed more slowly. These differences in egg production following the three patterns of exposure to infection passed almost unnoticed by the authors, yet the results illustrate clearly the different courses followed by the infections. They show that the rate of development of resistance is dependent on the nature of the exposure to infection.

Resistance has also been reported following infection with attenuated cercariae, where attenuation is effected by exposure of the animals to irradiated cercariae. Under these conditions the cercariae develop into adult worms but they do not become sexually mature. No eggs are laid and there is no appreciable pathogenesis.

Villella et al. (1961) reported resistance to challenge exposures of S. mansoni in mice previously exposed to irradiated cercariae, and Hsü et al. (1962) claimed to have developed absolute resistance to S. japonicum in some rhesus monkeys previously immunised with irradiated cercariae of the same species.

Erickson and Caldwell (1962) reported a considerable worm reduction from challenge exposures of rats previously exposed to irradiated S. mansoni cercariae, and Smithers (1962b) obtained similar results for the same parasite species in rhesus monkeys. Radke and Sadun (1963) demonstrated resistance in mice following immunisation with irradiated S. mansoni cercariae. Perlowagora-Szumlewicz and Olivier (1963) and Perlowagora-Szumlewicz (1964) doubted the full validity of the previously reported results because they found that the maturation of the challenge infection was not prevented but delayed. However, they also reported some degree of resistance in mice previously exposed to irradiated cercariae of S. mansoni. There is little doubt that resistance can result from exposure to attenuated cercariae but the thorough investigations of Perlowagora-Szumlewicz in 1964 show that experimental results must be interpreted with care. The bold claims of Hsü and his colleagues require confirmation.

The use of somatic or metabolic antigens to stimulate the development of resistance has also been attempted but with inconclusive results. Moore et al. (1963) obtained some indications of transient resistance in mice following injection of somatic antigen derived from S. mansoni eggs, but there was no significant decrease in the numbers of worms recovered after challenge infections. Vogel and Minning (1953) and Vogel (1958, 1962a) reported a total lack of resistance in rhesus monkeys previously inoculated with somatic S. japonicum antigens. Smithers (1962c) reported similar results with S. mansoni antigens in rhesus monkeys, as did Ritchie et al. (1962) with S. mansoni antigens in mice. However, Watts (1949) reported that intra-cutaneous inoculation of mice with somatic antigen from adult S. mansoni worms resulted in a significant decrease in numbers of worms recovered after

challenge infections, and Sadun and Lin (1959) reported that metabolic antigens inoculated into mice also resulted in a protection against challenge infection. Levine and Kagan (1960) demonstrated an increased survival time after challenge infection in mice previously inoculated with metabolic antigens derived from the cercariae and adult worms of S. douthitti. As suggested by Vogel (1958), the use of more concentrated metabolic antigens may give more conclusive results.

In order to demonstrate the presence of circulating protective antibodies, it is desirable to show a passive transfer of resistance where a non-immune animal is protected following the injection of serum from an immune animal. Vogel and Minning (1953), Stirewalt and Evans (1953) and Levine and Kagan (1960) all attempted this passive transfer of protection, without result. However, Sadun and Lin (1959), working with S. japonicum in mice, claimed to have demonstrated serological protection with passive transfer, and Bruce and Sadun (1964) reported that serum transfer from resistant rhesus monkeys, previously infected with S. mansoni, to rats resulted in significant reduction of numbers of worms maturing from challenge infections.

It has yet to be proved that resistance will result from passive transfer or from antigenic stimulation. It is particularly desirable to show passive transfer of protection if this is possible, because passive transfer indicates a resistance which results from circulating protective antibodies. The work of Sadun and Lin in 1959 indicated protection from passive transfer, but this has never been repeated. The subsequent work of Bruce and Sadun (1964) cannot be accepted as demonstrating passive transfer since they used the white rat for the experiment and this animal is highly refractory to S. mansoni.

The development of acquired resistance after living immunising infections has been conclusively demonstrated. Very little information is as yet available on the conditions of immunising infections which give rise to the strongest resistance. However, there have been some observations on the degree of resistance developed after single or multiple infections and there are indications that the

period of immunisation and the level of the immunising infection are important. There have been no reports of investigations designed to assess the importance of these factors which appear to influence the rate and degree of the development of resistance.

Immunisation infection experiments to demonstrate the development of acquired resistance in animals.

The phenomenon of resistance acquired by animals infected with bilharziasis has been clearly demonstrated by many workers but it was considered necessary to attempt to show, in laboratory animals, that the circumstances of the immunising infections influence the rate and degree of the development of resistance. The work of other authors has given some indications of this but these indications have been incidental, and there has been no attempt at quantitative assessment of the degree of resistance developed under different circumstances of immunising infection.

Because of the difficulties in obtaining laboratory hosts of S. capense in sufficient numbers to give valid data on resistance, the experimental immunisation-challenge infections have been limited to infections of S. mansoni in mice. All the cercariae used for infections were derived from laboratory bred and infected B. pfeifferi snails. Except in the series using immunising infections of one sex only, the cercariae for each experiment were drawn from a pooled source derived from at least ten snails in an attempt to ensure that both male and female cercariae were present. A minimum of 30 mice were used for any group of any series, and all the mice were between one and two months old at the times of first exposure.

The observations of other workers indicate that there are four factors of importance which influence the degree and rate of development of resistance in animals as well as in man. These factors are:-

(a) the numbers of cercariae of the immunising exposure;

(b) the period for which the immunising infection is present in the animal before it is subjected to challenge exposure;

(c) the pattern of exposure to the immunising infections;

(d) the presence or absence of eggs from the worms of the immunising infections. This last factor was believed to be important because Olivier-Gonzalez (1954) had demonstrated that eggs were very strongly antigenic. The reports of several workers, including Kagan (1952), Meleney and Moore (1954) and Smithers (1962a) also indicated that strongest resistance resulted from bisexual infections.

It was decided therefore to attempt to show that these four factors do in fact influence the development of acquired resistance. Using a procedure involving immunising and challenge infections, these four factors were examined in comparison with a predetermined standard arrangement of infections which had been shown, in preliminary experiments, to give rise to strong resistance. This standard arrangement of infections involved a single subcutaneous immunising exposure to 50 cercariae of both sexes, followed after a period of six months by a single percutaneous challenge exposure to 250 cercariae of both sexes. The mice were killed, examined and perfused two months after the challenge exposure.

Subcutaneous injection was chosen for the immunising infection because data from preliminary experiments indicated that subcutaneous exposure, of all the methods of infection, gave the most uniform recovery of worms maturing from infections. These preliminary observations were confirmed by Gönnert (personal communication). Since it has been indicated (von Lichtenberg and Ritchie, 1961) that many cercariae of challenge exposures are killed in the skin in resistant animals, it was considered necessary to use the percutaneous route of infection for all challenge exposures, to allow maximum opportunity for resistance to influence the course of the infection.

All worms recovered from the mice were separated into two groups

according to sex, counted and measured for length. A weighed portion of liver from each mouse in the first experimental series was crushed between two glass slides and examined through a microscope fitted with a source of ultra-violet light. The eggs in the liver were clearly visible, having a bright orange autofluorescence which contrasted with the dull green autofluorescence of the liver tissue, and they were easily counted. Estimates were thus made of the numbers of eggs per gram of liver.

For each variation in exposure pattern, number of immunising cercariae, or immunising period in the immunisation-challenge experiments, a control group of mice was exposed to similar infection for the same period. The control group was killed without challenge exposure. These control groups were termed the immunised controls. A large group of mice received the equivalent of the challenge exposure without previous immunising exposure, and these were killed, examined and perfused two months after the exposure. This control group was termed the challenge control. It was intended that there would be parallel challenge controls in all experimental series, but the numbers of mice available precluded this arrangement and a single series of over 100 challenge control mice had to be accepted for all experiments.

In the interpretation of the significance of the results of perfusion for the number of worms maturing from any infections, the transformation $\sqrt{n_i + 1}$ has been used, where n_i is the number of worms recovered from each mouse. The reasons for the use of this transformation are given in the appendix (page 145)

The effects of different patterns of exposure on the development of resistance in the mice were examined in four experimental series, each designed to examine one of the four factors considered to be of importance in the development of resistance.

Series 1.

This experimental series involved varying the level of the immunising exposure, with all other conditions being kept to the standard

arrangement. Three groups of 30 mice were used. In the first group, 1A, each mouse was injected with 50 cercariae, according to the standard arrangement for immunising and challenge infections, and the results from this group were used for comparison in subsequent experimental series. The mice of group 1B received single immunising exposures of 25 cercariae, and those of group 1C received 100 cercariae. All groups were challenged after six months to 250 cercariae by percutaneous infection, and killed after a further two months.

The results for this series, giving the numbers of worms recovered and the relevant square root transformation analyses, are given in Table 2. Table 3 gives the comparison between these analyses and those of the challenge controls. Since the immunised and challenged mice were exposed to greater total numbers of cercariae than the challenge controls, it was necessary to introduce corrections based on the results of the immunised cobtrol groups. The derivation of these corrections is discussed in the appendix 1 (page 145). The results for the challenge control and the immunised control groups are presented in Table 4.

The results show that the difference between the transformation means of group 1A originally exposed to 50 cercariae, with an immunisation period of six months, and the challenge control group, was 2.03. The standard error of difference was 0.48, showing a significant decrease in worm numbers from the challenge exposure in the immunised mice.

The difference between means of group 1B, exposed to an immunising infection of 25 cercariae, and the challenge control group was 1.6, with a standard error of difference of 0.40, again showing a significant reduction in worm burden. For group 1C, exposed to 100 immunising cercariae, the difference between the means was 2.22 but the high standard error of difference of means of 1.68 detracted from the significance of this difference.

When comparing groups 1A, 1B and 1C of this first series, the corrected means showed no statistically significant difference in

TABLE 2.

Series 1. Effects of worm load on the development of resistance. Results of perfusion of mice exposed to three levels of immunising infection of S. mansoni, challenged after six months, and killed and perfused after a further two months.

	Group A	Group B	Group C
Number of mice exposed to immunising infection	30	30	30
Number of cercariae used for immunising infection	50	25	100
Number of mice surviving to date of challenge exposure	14	19	11
Number of mice surviving to date of perfusion	12	19	6
Total number of worms recovered	201	534	184
Sum of $\sqrt{n_i + 1}$, where n_i is the number of worms recovered from individual mice	47.17	98.08	25.01
Mean value $\sqrt{n_i + 1}$	3.93	5.16	4.17
Standard deviation $\sqrt{n_i + 1}$	± 1.59	± 1.62	± 4.10
Range of values of $\sqrt{n_i + 1}$	1.00-7.21	1.00-8.49	1.00-10.59

TABLE 3.

Comparison of results of perfusions of Series 1 mice and controls.

	Group A	Group B	Group C
Mean of $\sqrt{n_i + 1}$ for test groups, where n_i are the numbers of worms recovered from individual mice	3.93	5.16	4.17
Correction for immunised controls	1.10	1.91	1.53
Corrected mean for test group	2.83	3.25	2.64
Mean of $\sqrt{n_i + 1}$ for challenge controls	4.86	4.86	4.86
Observed difference between means	2.03	1.61	2.22
Standard error of difference between means	0.48	0.38	1.68

TABLE 4.

Control Groups: Results of perfusion of mice in immunised control and challenge control groups.

	Challenge Control	Immunised Control Groups		
		I	II	III
Number of mice exposed	110	30	30	30
Number of mice surviving to date of perfusion	101	24	15	9
Total number of cercariae used	250	50	25	100
Number of exposures	1	1	1	1
Number of cercariae used for individual exposures	250	50	25	100
Time in weeks separating exposures	-	-	-	-
Time in months between first exposure and perfusion	2	8	8	8
Number of worms, male and female, recovered	2506	168	90	158
Sum of $\sqrt{n_i + 1}$, where n_i are the numbers of worms recovered from individual mice	490.99	65.18	37.40	37.18
Mean of $\sqrt{n_i + 1}$ (m)	4.86	2.72	2.49	4.13
Standard deviation of $\sqrt{n_i + 1}$ (S.D.)	± 1.48	± 0.81	± 0.29	± 1.30
Range of values of $\sqrt{n_i + 1}$	1.00-9.38	1.41-4.69	1.41-4.69	2.24-7.00
Correction for immunised controls (m - 2 S.D.)	-	1.10	1.91	1.53
Experimental series for which correction is used	-	1A,2A,B,C,4	1B	1C

numbers of worms recovered although there was an indication of greatest reduction in group 1A.

Series 2.

The second series was designed to evaluate the effects of varying the period between the immunising and challenge exposures.

In this series, two groups of 30 mice were exposed to 50 cercariae each. Group 2A was challenged after three months and group 2B after nine months, and these two groups were compared with the challenge control and with group 1A of the first series, in which the mice were also exposed to an immunising infection of 50 cercariae, but they were challenged after six months. The results for the two groups of this series are presented in Table 5. Table 6 gives the comparisons between these results, with the corrected means, and the results of the challenge controls.

The difference between the transformation means of group 2A and the challenge control was 2.09. The standard error of the difference was 0.37, indicating a significant decrease in numbers of worms maturing from the challenge infection. The difference between the means of group 2B and the challenge control was 2.06, with a standard error of 0.60, again showing a significant reduction in worm burden. There were no significant differences between the means of groups 2A and 2B of the second series, or between either of these groups and group 1A of the first series.

Series 3.

In the third experimental series an attempt was made to compare the resistance developed as a result of immunising exposures of different patterns. Three groups of 60 mice were exposed to infection; the mice of group 3A each received five exposures to only ten cercariae for each exposure, with 14 day intervals between the exposures; group 3B received two exposures of 25 cercariae with an interval of 28 days between exposures; group 3C received two exposures of 50 cercariae, again with an interval of 28 days between

TABLE 5.

Series 2. The influence of duration of immunising infection on the development of resistance. Results of perfusion of mice exposed to 50 cercariae, and challenged after three months or nine months to 250 cercariae. The results from Group 1A are included for comparison.

	Group 2A	Group 2B	Group 1A (from Table 2)
Number of mice exposed to immunising infection	30	30	30
Period of immunising infection, in months	3	9	6
Number of mice surviving to date of challenge exposure	26	18	14
Number of mice surviving to date of perfusion	18	7*	12
Total number of worms recovered	117	114	201
Sum of $\sqrt{n_i + 1}$, where n_i represents the number of worms from each mouse	69.58	27.32	47.17
Mean value of $\sqrt{n_i + 1}$	3.87	3.90	3.93
Standard deviation of $\sqrt{n_i + 1}$	± 1.46	± 1.55	± 1.59
Range of values of $\sqrt{n_i + 1}$	1.00-7.14	1.00-6.08	1.00-7.21

* = 4 mice killed in error, (of original 11).

TABLE 6.

Comparison of results of perfusion of Series 2 mice and controls.

	Group 2A	Group 2B
Mean of $\sqrt{n_i + 1}$ for test groups, where n_i represents the numbers of worms for each mouse	3.87	3.90
Correction for immunised controls	1.10	1.10
Corrected mean for test group	2.77	2.80
Mean for challenge controls	4.86	4.86
Observed difference between means	2.09	2.06
Standard error of difference	0.18	0.60

exposures. Three months after the first exposures, the surviving mice of each group were divided equally into two lots, one of which was subjected to challenge exposure of 250 cercariae, and the other was left without challenge as the immunised control. The challenged mice were killed, examined and perfused two months after the challenge exposure. The immunised controls were killed at the same time. The results of the challenge groups are given in Table 7, and Table 8 gives the comparisons between these results, with the corrected means, and the results of the challenge controls. Table 9 gives the results of the immunised control groups for this series.

In the groups 3A and 3C there were no significant reductions in the numbers of worms recovered after the challenge exposures. However, in group 3B, which received 25 cercariae for each of two immunising exposures, there was a significant reduction in worm numbers recovered after challenge. The difference in the transformation means of this group and the challenge control was 1.13, with a standard error of difference of 0.20. Group 1A of the first series which received a single immunising exposure of 50 cercariae, showed a greater reduction of worm burden than group 3A, which received the same total number of cercariae divided over five different exposures. The difference in the transformation means was 1.54 with a standard error of difference of 0.66. There was also an indication of greater reduction in group 1A than in group 3B, which received two exposures of 25 cercariae, but the difference in the means was not fully significant. There was an indication, which was not verified statistically, of a greater reduction in worm burden in mice of group 1C, immunised with a single exposure of 100 cercariae, than in the mice of group 3C immunised with the same total number of cercariae in two exposures of 50 cercariae for each exposure. In this case the difference in the means was 1.70, but the low numbers of surviving mice, and the large number of worms (112) recovered from one mouse in group 1C, resulted in a very high value for the standard deviation within that group. Consequently there was a high standard error of difference between the means of the two groups of 1.72.

TABLE 7.

Series 3. The effects of different exposure patterns on the development of resistance. Results of perfusion of three groups of mice exposed under different conditions and challenged after three months to 250 cercariae. Results from Group 2A are included for comparison.

	Group 3A	Group 3B	Group 3C	Group 2A (From Table 5)
Number of mice exposed to immunising infection	30	30	30	30
Total number of cercariae used	50	50	100	50
Number of immunising infections	5	2	2	1
Number of cercariae for each exposure	10	25	50	50
Intervals, in weeks, between exposures	2	4	4	-
Number of mice surviving to date of perfusion	18	19	20	18
Total number of worms recovered	436	351	489	117
Sum of $\sqrt{n_i + 1}$, where n_i represents the number of worms recovered from each mouse	86.34	83.15	94.73	69.58
Mean value $\sqrt{n_i + 1}$	4.80	4.38	4.74	3.87
Standard deviation of $\sqrt{n_i + 1}$	1.53	0.58	1.77	1.46
Range of values of $\sqrt{n_i + 1}$	2.24-6.93	3.74-6.08	2.00-9.27	1.00-7.14

TABLE 8.

Comparison of results of perfusion of Series 3 mice and controls.

	Group 3A	Group 3B	Group 3C
Mean value of $\sqrt{n_i + 1}$ for test groups, where n_i represents the number of worms for each mouse	4.80	4.38	4.74
Correction for immunised control	0.43	0.65	0.40
Corrected mean for test group	4.37	3.73	4.34
Mean for challenge controls	4.86	4.86	4.86
Observed difference between means	0.49	1.13	0.52
Standard error of difference	0.39	0.20	0.42

TABLE 9.

Series 3. Results of perfusion of mice of immunised controls.

	Immunised control groups			
	3A (from Table 4)	3B	3C	3D
Number of mice exposed	30	30	30	30
Number of mice surviving to date of perfusion	24	19	20	25
Total number of cercariae used	50	50	50	100
Number of exposures	1	5	2	2
Number of cercariae used for individual exposures	50	10	25	50
Time, in weeks, separating exposures	-	2	4	4
Time, in months, between first exposure and perfusion	8	5	5	5
Total number of worms, male and female, recovered	168	47	39	53
Sum of $\sqrt{n_i + 1}$ where n_i is the number of worms recovered from individual mice	65.18	33.30	32.91	41.47
Mean of $\sqrt{n_i + 1}$ (m)	2.72	1.75	1.65	1.66
Standard deviation of $\sqrt{n_i + 1}$ (S.D.)	± 0.81	± 0.66	± 0.50	± 0.63
Range of values of $\sqrt{n_i + 1}$	1.41-4.69	1.00-3.00	1.00-2.65	1.00-2.65
Correction for immunised controls (m - 2 x S.D.)	1.10	0.43	0.65	0.40

Series 4.

The fourth and final experimental series was designed to determine if a single sex immunising infection would stimulate the development of resistance in mice. Thirty mice were exposed to 50 cercariae each, as a single immunising exposure. Each mouse received cercariae from one snail only. The snails were previously exposed individually to only one miracidium each. Since the sex of the cercaria is determined on the sex of the miracidium, this ensured that each mouse received an immunising infection of only one sex. Additional mice were exposed to the cercariae from each snail. These were killed without challenge infection to verify that only one sex of worm was present. The experimental mice were challenged after six months to 250 cercariae of mixed sexes, and killed and perfused two months after this challenge infection. The results of this fourth series are presented in Table 10. There was a slight, but statistically significant reduction in worm burden resulting from the challenge infection; this difference between the transformation means was 0.57, with a standard error of 0.25.

It was stated earlier that all the worms recovered by perfusion were separated into two groups according to sex, and that they were measured for length. No statistically significant differences in the numbers of worms of each sex maturing from any of the challenge series could be detected, and there was no evidence of stunting of surviving worms. It has also been stated that estimates were made of the numbers of eggs per gram of liver for all the mice in the three groups of the first series, and for the mice of the challenge control group. There was no apparent decrease in the numbers of eggs in the livers of the immunised mice.

Conclusions from animal infection experiments.

These small series of infection and challenge experiments using S. mansoni in mice give some indication of the important influence which the pattern of immunising infection bears on the subsequent development of resistance. Over 700 mice were used in the four

TABLE 10.

Series 4. The results of immunising infections of one sex on the development of resistance in mice. Comparison with Group 1A from Series 1 of numbers of worms recovered after challenge exposures to 250 cercariae of both sexes.

	Test Group 4	Group 1 A
Number of mice exposed to immunising infection	30	30
Number of cercariae used	50 (males only)	50 (both sexes)
Period of immunisation, in months	6	6
Number of mice surviving to date of perfusion	23	12
Total number of worms recovered	664	201
Sum of $\sqrt{n_i + 1}$ where n_i represents the number of worms recovered from each mouse	124.00	47.17
Mean value $\sqrt{n_i + 1}$	5.39	3.93
Standard deviation of $\sqrt{n_i + 1}$	0.92	1.59
Corrected means (correction from immunised control)	4.29	2.83
Observed difference between means of test mice and challenge control	0.57	2.03
Standard error of difference	0.25	0.48

experimental series, but this number was only sufficient to indicate that different patterns of exposure influence the development of resistance. However, the results indicate that it would be possible to obtain quantitative assessment of the rate and degree of development of resistance under different conditions of exposure.

The most important facts which are apparent from the results are the great variations in individual susceptibility to infection, and the variations in the development of resistance in individual mice. In all the experiments there were marked differences in the numbers of worms recovered, despite the carefully standardised procedures of exposure. Examples of this can be taken from the challenge control group where 87 worms were recovered from one mouse, but no worms from another, and from the immunised control group exposed to a single infection of 50 cercariae, where only one worm was recovered from one mouse, and 21 from another. These two examples illustrate the differences essentially of susceptibility. Examples of differences in initial susceptibility, and probably, in host responses, can be taken from group 1A, with worm recoveries from individual mice varying from nil to 51, and from group 1C, where the variations were from nil to 112.

In drawing deductions from the results of all these experiments, it must always be remembered that the results are taken only from the mice surviving to the date of perfusion. The mice with the least resistance and greatest susceptibility die before they can be examined. However, the mortality after the challenge infections was generally low, and the deaths occurring between immunising and challenge infections were compensated by the deaths in the immunised controls.

It is apparent that, in mice infected with S. mansoni, the most important single factor exerting an influence on the development of resistance is the number of cercariae used for the immunising infection. In this series a single immunising exposure to 50 cercariae resulted in the most significant resistance. A light infection will develop resistance either more slowly, or to a lesser extent. The exposure of group 1B to 25 cercariae, with an immunising period

of six months, resulted in resistance, but to a lesser degree than the exposure of group 1A to 50 cercariae for the same immunising period. A very heavy immunising exposure, as in groups 1C or 3B results in a high mortality, and there is also an indication of immunological paralysis, where the development of resistance is limited by the weakening of the animal by the disease resulting from the heavy infection.

The results of these experiments indicate that greater resistance to superinfection is developed in animals receiving a single initial immunising exposure than in animals receiving two or more exposures with the same total number of cercariae. This is contrary to the results of Thompson (1954), Lurie and de Meillon (1957) and Kagan (1958) who found that repeated light infections in animals resulted in the most appreciable resistance. It is also contrary to the assumptions made by Manson-Bahr (1958) that repeated light infections in man would give rise to greatest resistance. It is not contrary to the conclusions drawn by Gerber (1952) who, reporting on the epidemiology of S. haematobium infections in man in Sierra Leone, suggested that maximum protection was conferred on people receiving a moderate initial exposure, followed later by regular re-exposure. Gerber maintained that, to achieve this protection, the intervals between the initial and subsequent exposures should extend over a season with no transmission of infection.

The results indicate that resistance is developed very rapidly in mice since there was significant resistance in group 2A, exposed to 50 cercariae and challenged after only three months. The differences in the degree of resistance resulting from exposure to 50 cercariae in the three groups 2A, 1A and 2B, in which the immunising periods were three, six and nine months respectively, were insignificant, and it must be concluded that the duration of the immunising infection is not as important a factor as the worm burden in its influence on the development of resistance. This contradicts the results of Ritchie et al. (1963) who reported strong resistance in small laboratory animals after six months of infection, but a decreasing resistance

after longer periods of 12 months or more.

Single sex infections gave very poor resistance response although there was a slight decrease in the numbers of worms maturing from challenge exposures.

There was no evidence of stunting of worms of the challenge infections of immunised mice, and there was no apparent difference in the effects of resistance on the two sexes of worms. The methods used were not sufficiently sensitive to detect any differences in the numbers of eggs in the livers of resistant and non-resistant mice.

These four series of experimental infections in mice confirm the reports of other workers that, following on initial infections, providing that these are neither too light nor too heavy, there is developed in mice a protection which manifests itself as a resistance against subsequent challenge infections. These series also indicate the important influence exerted on the development of this protection by the nature of the initial immunising infection or infections. The majority of authors suggest that the mouse, unlike the rhesus monkey, only develops a partial protection against either re-infection or superinfection, and, as discussed later, the same is possibly true for man. Although close analogies are not permissible, it is possible that man reacts in a similar manner to differences in the nature of the initial infections.

PART III.

EVIDENCE OF THE DEVELOPMENT, IN MAN, OF ACQUIRED RESISTANCE TO INFECTIONS OF SCHISTOSOMA SPP.

Kagan (1958) quotes the generally accepted classification of immune states listed by Boyd (1956), outlining this classification as follows:

I. Innate Immunity (constitutional or racial).

II. Acquired Immunity.

1. Active - (a). Infection (natural)
   (b). Vaccination (artificial)
2. Passive - (c). Natural (congenital)
   (d). Artificial (serum transfer).

The epidemiological data discussed later indicates the occasional existence of innate immunity in man, but it is insufficient to warrant any definite conclusion. Since the degree of such immunity would probably not be affected by differences in extent and nature of exposure, further consideration of innate immunity is not required for the present investigation. It is probable that natural, passive immunity does occur, since some individuals can live in an endemic area without ever showing any indications of infection, although they are undoubtedly often exposed to infection. In such people, even repeated parasitological and serological tests fail to detect any signs of infection.

There is as yet no definite evidence of artificial immunity, either by vaccination or by serum transfer.

Further consideration will therefore be given only to active acquired immunity resulting from infection.

If it can be accepted that the development of resistance is an active process, then it is necessary to investigate the nature and extent of the effects of this resistance; it is not necessary, for the purposes of the present investigation, to examine the mechanism and processes leading to the development of the resistance. The natural ideal situation for the survival of the parasite species

would be the development of a degree of resistance which would protect the host from a continued accumulative invasion by parasites which would eventually kill the host and, therefore, its parasites. At the same time it would not be to the advantage of the parasite species if the degree of resistance was great enough to overcome both the initial infection and any subsequent re-infections. In practice it would appear that the degree of resistance will vary with different conditions of immunising infection, and, as with many of the bacterial or virus diseases, available evidence indicates that there is a considerable variation in resistance response between individuals. In addition, it appears that even in highly resistant individuals the acquired resistance can be overcome in certain circumstances. Such circumstances include the general weakening of body resistance by concurrent illness, and exposure of the infected individual to overwhelmingly heavy infection.

Vogel (1958) stated that "experimental re-infection 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." However, even if it proved possible to conduct a series of human experiments involving challenge infections following controlled initial immunising infections, quantitative interpretation would be difficult without surgical examination to determine worm numbers and activity. This is clearly impossible and the best that can be expected from human experiments is a qualitative assessment of acquired resistance. At present it is necessary to depend on the limited direct evidence from two small and inconclusive series of human challenge exposures for the presumption of the development of acquired resistance, and on an extensive range of indirect evidence for the quantitative evaluation of the importance of this resistance.

The indirect evidence is derived from:

(a) the presumption that immunogenic processes are active in infected persons since antibodies can be detected in these people;

(b) from the interpretation of the results of the epidemiological investigations, which demonstrate a pattern of distribution of the disease in relation to age which can only be explained by acceptance of the concept of acquired resistance, and

(c) conclusions based on analogy between animals and man.

Human Volunteer Experiments to Demonstrate Active, Acquired Resistance.

Very few human volunteer experiments to demonstrate the development of active acquired resistance resulting from infections have been reported in the literature, and of these only the work of Fisher (1934) furnishes direct evidence of the development of this resistance; a further small series of two attempts to infect human volunteers was undertaken in Rhodesia, and the results of this series supports the results reported by Fisher.

Fisher noted that the peak of prevalence of S. intercalatum infections in the African communities of certain villages occurred between the ages of 5 and 15, and that there was a marked falling-off in the prevalence with increasing age to the extent that no infections were detected in persons over 35 years old. He argued that the fall-off was the result of the development of resistance, and, in an endeavour to further demonstrate this, he exposed six volunteers to infection. All six were males between the ages of 35 and 40, and all were fishermen with the implied history of frequent contact with infected water. In each case stool specimens were examined twice but no eggs were found. The men were exposed to mixed freshly shed cercariae from a number of snails; exposure was by total immersion of the arm for 15 minutes. At the same time mice were exposed to

cercariae from the same source, and at subsequent autopsy these mice were found to be harbouring S. intercalatum worms of both sexes. After exposure the men complained of pruritis but none showed any illness. Stool specimens from each volunteer were examined at 3 months and 6½ months after exposure but no eggs were found. After 8 months three of the men were found to be passing a few eggs in the faeces, but repeated examinations of the other three were negative. Some time later (the period was not specified) five of the men were traced and again examined; in each case a careful search failed to reveal any eggs. Fisher emphasises that at no stage was any clinical manifestation of the infection apparent in any of the men.

This experiment demonstrated that these six men, even those who had passed a few S. intercalatum eggs, were resistant to infection. However, S. intercalatum is not a common species and it is limited in its distribution to certain areas of the former Belgian Congo. It has yet to be demonstrated that man is the definitive host for this species which is possibly synonymous with S. mattheei. If this is the case it is difficult to accept the results of the experiment as applying also to essentially human parasites such as S. capense or S. mansoni.

A similar criticism may also be made to the series of two human volunteer exposures to infection carried out in Rhodesia. Two volunteers, one a man aged 26 (V.C.) and the other a woman aged 24 (Z.L.), were exposed to large numbers of cercariae derived from a number of "wild" infected Bulinus (Physopsis) globosus snails; at the same time, two baboons (Papio ursinus) were also exposed to cercariae from the same source. The two volunteers had had frequent exposure to infected water throughout their lives, but neither had any history of previous infection, in spite of repeated examinations of urine and stool specimens.

Neither of the volunteers passed any eggs during a period of observation lasting over one year after exposure, but the baboons, however, were found at autopsy approximately 6 months after exposure

to be harbouring worms which were identified by Alves (personal communication) as S. haematobium and S. mattheei. This identification is suspect, and it is possible that all the worms were S. mattheei. This experiment was undertaken prior to the publication of the paper by le Roux (1958) on the species of Schistosoma and the designated S. haematobium by Alves would refer to the southern African species S. capense. Prior to le Roux's report, the two species were regarded as synonymous.

The human volunteers each immersed the forearm and elbow for one hour in water containing approximately 3,000 cercariae. Each experienced a pronounced and unpleasant dermatitis which appeared before the full period of the exposure had expired, and which gradually subsided over a period of two weeks. Prior to exposure, both volunteers had been repeatedly tested for infection by specimen examinations, but no eggs were found. No eggs were ever found in the excreta of the baboons. The two volunteers were also tested by the intradermal test, with negative results, and differential blood counts showed less than 4 percent eosinophil cells. Specimen examinations, intradermal tests (using antigen of cercarial origin) and further differential white cell counts were undertaken each week after exposure for a period of 13 weeks on the male volunteer, and 18 weeks on the female volunteer; thereafter, specimen examinations were done at approximately one month intervals for over a year. At no time were eggs of any species of Schistosoma found. Table 11 gives the results of intradermal tests and the differential white cell counts. It will be seen that the male volunteer gave a positive skin test reaction from the third week, and that both demonstrated an increased eosinophilia from the third to the sixth week after exposure, subsiding to 4 percent or less after eight weeks. In the male, however, a sudden increase in eosinophilia took place in the 12th week, and this corresponded to a return of a positive skin test reaction. During this two-week period the person suffered from an attack of enteritis with severe diarrhoea.

TABLE 11.

Results of Intradermal tests and eosinophil counts in two human volunteers experimentally exposed to infection. The results are given for the day of exposure and thence for intervals of one week for 18 weeks after exposure.

	Male Volunteer (V.C.)		Female Volunteer (Z.L.)	
	Intradermal test	Eosinophilia (per cent)	Intradermal test	Eosinophilia (per cent)
Exposure date (7.2.1952)	negative	3	negative	4
1st week after exposure	negative	3	negative	6
2nd " " "	negative	4	negative	8
3rd " " "	positive	8	negative	10
4th " " "	positive	8	negative	15
5th " " "	positive	8	negative	9
6th " " "	positive	7	negative	8
7th " " "	doubtful	3	negative	3
8th " " "	doubtful	4	negative	4
9th " " "	doubtful	3	negative	4
10th " " "	doubtful	7	negative	3
11th " " "	positive	11	negative	2
12th " " "	positive	4	negative	3
13th " " "	not done	not done	negative	5
14th " " "	not done	not done	negative	4
15th " " "	not done	not done	negative	4
16th " " "	not done	not done	negative	4
17th " " "	not done	not done	negative	less than 4
18th " " "	not done	not done	negative	less than 4

These experimental infections indicate that although the parasites undoubtedly entered, as evidenced by the dermatitis, they probably failed to mature; in any case, no eggs were found. Newsome (1956), Vogel (1958) and others have indicated that the parasites in resistant animals face maximum mortality in the period between two weeks and three weeks after exposure, and the rise in the eosinophil count in the two volunteers is consistent with this. It should be added that one of the volunteers (Z.L.) subsequently was exposed to S. mansoni cercariae under natural conditions, and she contracted the disease. The other volunteer (V.C.), in spite of repeated exposure to cercariae under natural conditions, which must have included cercariae of both S. mansoni and S. capense, has never been found to be passing eggs of either species of Schistosoma.

This series is even less conclusive than that of Fisher, but again there is a demonstration of resistance to schistosome infection. The fact that one of the volunteers subsequently contracted an infection of S. mansoni is in no way contradictory to the concept of acquired resistance, since there is evidence that protective resistance is, at least to some extent, species specific in action.

Further direct evidence of resistance is given by the records of individuals in which the resistance apparently persists after chemotherapeutic treatment. Government employed irrigation engineers and field workers in Rhodesia are compelled by the nature of their duties to suffer repeated exposure to infection, since they have contact with water known to be infected. Many of these workers are thoroughly tested for bilharziasis, by repeated specimen tests, each year, and if any are found to have contracted the disease, they are immediately treated for it, usually with a drug having an antimony base. There are records of several of them who, despite repeated exposure, have never developed active infections. Two others, recruited 10 years previous to the last time they were examined, showed signs of chronic infections when they were first examined shortly after they were recruited. They received treatment and, although they were repeat-

edly exposed to re-infection during the next 10 years, they have never again been found to be passing eggs, nor has there been any clinical sign of the redevelopment of active infection.

The direct evidence, from human infections, of resistance is insufficient to warrant a firm conclusion that resistance to bilharziasis is acquired by man. This information can be supported by the indirect evidence that antigen-antibody reactions occur, showing that immune responses in the host are possible, and also by epidemiological evidence.

Antigen-antibody Reactions in Immunological Tests as Probable Evidence of Parallel Protective Responses.

It has yet to be proved that a person with a parasitic infection can develop circulating antibodies which will afford a protection against the parasite. However, there are circulating antibodies in the serum of such an infected person, and these antibodies can be detected by serological tests. These detectable antibodies are not necessarily protective in action. The distinction must therefore be made between detectable and protective antibodies. Although the presence of detectable antibodies in serum does not constitute proof of the concurrent existence of protective antibodies, they are produced by the same stimulus. Evidence of the presence of detectable antibodies indicates that the host is capable of an immune response to the presence of the parasite. The presence of detectable antibodies therefore indicates the probability that the host is able to produce protective antibodies.

A large number of diverse diagnostic techniques have been developed to detect the circulating antibodies resulting from a parasitic infection, and generally these techniques are but minor modifications of tried and proven immunodiagnostic techniques for the identification of bacterial or virus diseases. The fact that these techniques can be used to detect infections in man of any of the species of Schistosoma is evidence of immunological activity resulting from schistosome infection. If there is immunological

activity, it is logical to assume that protective responses to the infection are possible.

The potential value of immunodiagnosis was early recognised. Yoshimoto (1910) demonstrated the feasibility of serological diagnosis of S. japonicum infections in man with a small series of satisfactory complement fixation tests using as antigen an alcoholic extract of adult worms. Fairley (1919) used a saline extract of crushed livers from snails infected with either S. mansoni or S. haematobium. He found that either antigen gave good complement fixation results for the detection of antibodies in the sera of patients infected with either species, or even of other species. Le Bas (1922) believed the antigen to be associated with protein but Fairley (1925) claimed that the antigen was lipoidal in origin. Neither author recognised that both components might be antigenic.

Antigens for use in immunodiagnostic techniques have been derived from every stage in the cycle of the schistosome worm, and even from the excretions and secretions of the different stages. In addition to this, antigens have been prepared in different ways and successful reactions have been obtained using different fractions of high purity derived from both protein and non-protein components of originally crude extracts of the parasites. This indicates the complexity of antigenic fractions which can be derived from any crude extract of adult worms or of other stages of the cycle.

Considerable attention has been given to the specificity of the immunodiagnostic responses. Some authors have claimed to have observed species specificity in that stronger responses are apparent between antigens and their homologous antibodies, whereas reactions between antigens of one species and antibodies of another are weaker although clearly demonstrable. Hsü and Hsü (1961b) claim to have shown differences even between geographic strains of the same species - S. japonicum. Most authors stipulate an intrageneric relationship in antigen-antibody response, but several have reported cross reactions between the genus Schistosoma and other genera, including

Fasciola, reported by Hassan and Betashe (1934), Heterophyes, reported by Sherif (1962), and Trichinella, reported by Anderson (1960).

In the course of this study, antigens were prepared according to the methods of Melcher (1943) from adult worms of both Fasciola gigantica and Schistosoma mansoni. Both antigens were used simultaneously for intradermal tests in a series of 6 volunteers. The correlation of results was complete not only in the 5 individuals giving skin reactions, but also in the one person who showed no reaction.

It is logical to assume that a crude antigen, with cross reactions between related genera, in fact may have components which show species specificity. Anderson et al. (1963) have reported on cross absorption studies with S. mansoni and Trichinella spiralis. They employed antigens adsorbed on cholesterol-lecithin crystals, with which they absorbed homologous antibodies from sera of people infected with either S. mansoni or T. spiralis. Although cross reactions took place with unabsorbed sera, genus specific reactions were reported following the absorption. This implied that the antibodies produced as a result of infection of either species were of at least two groups. The first were common antibodies which reacted with antigen of either species, and the second group of antibodies were specific in reaction with antigens. Absorption, with S. mansoni antigen, of the common antibodies in a serum from a patient infected with T. spiralis removed the common antibodies, and left only antibodies which were specific for T. spiralis. Similarly, absorption of common antibodies from sera of people infected with S. mansoni left antibodies which would react only with the S. mansoni antigen. This demonstrated that different antibody fractions are present in serum of an infected person.

The fluorescent antibody test for bilharziasis, extensively used for epidemiological surveys in the course of this study, was described by Sadun et al. (1960) and Cookson (1963). It is perhaps

the most sensitive and reliable of serological techniques for the diagnosis of bilharziasis, comparing for sensitivity only with the complement fixation test. The use of minute quantities of dried blood, collected on filter paper (Anderson et al. 1961a and Sadun et al. 1961a) and the use of preserved and counter-stained cercariae (Anderson et al. 1961b) add very greatly to the practical usefulness of the test.

Cookson (op. cit.) described the use of both the direct and the indirect fluorescent antibody techniques. In the direct method, which is impractical for routine use, the test serum is conjugated with a fluorescent stain; after subsequent incubation with the appropriate particulate source of antigen, the reaction can be detected by observing a coating of the antigen source by the fluorescent antibody. In the indirect or sandwich technique, which is suitable not only for routine diagnosis in individuals, but also for epidemiological surveys, the test serum is reacted with the antigen and the reaction is detected by the subsequent use of conjugated fluorescent anti-human antiserum.

The results of a series of comparative diagnostic trials undertaken in three communities in Rhodesia, in which the reliability of the fluorescent antibody test was assessed in people who were also thoroughly tested for infection by stool and urine examinations, are summarised below:

A total of 666 people were tested by both methods; of these 307 (46 percent) were found to be passing eggs of either S. mansoni or S. capense or both. Of these latter known infected people, 29 (9 percent) gave negative results with the fluorescent antibody test, and 278 (91 percent) gave positive results. 359 people were not found to be passing any eggs, although failure to find eggs in the stool or urine does not preclude the possibility of active infection. Of these 359 people, 198 (55 percent) gave negative, and 161 (45 percent) gave positive fluorescent antibody results. The results of the fluorescent antibody tests therefore corresponded to those of the specimen examinations in 476 (71.5 percent) of the 666 people

tested, since there was a negative correlation in 198 people and a positive correlation in 278 people. The results of the fluorescent antibody tests and specimen examinations failed to correlate in 190 (28.5 percent) of the people.

The greatest apparent error lies in the 161 (45 percent) of 359 people who gave positive fluorescent antibody test results but who were not found to be passing any eggs. The proportion of people in this category has been found to increase with age; this is believed to be an effect of the development of resistance, and the data to support this belief are presented later in this section.

A slide flocculation reaction was reported by Anderson (1960) when he employed washed cercarial antigen adsorbed on cholesterol-lecithin crystals. The test is reported to be highly sensitive. A modification of this test was described by Sadun et al. (1963) in which a very rapid test could be done under field conditions, on special cards requiring only three drops of blood derived from a finger prick. In the course of the present investigation, an attempt was made to use this plasma card test for epidemiological surveys. 153 people were tested by this method and also by urine and stool examinations for eggs of S. capense or S. mansoni. 99 people were definitely infected with bilharziasis since eggs were found in the specimens; of these, the plasma card test failed to detect 27 (27 percent). 54 people were not found to be passing eggs; of these, 31 (57 percent) gave positive flocculation of the antigen-carbon suspension, and 23 (43 percent) failed to do so. Because of the indefinite results, the use of the test was discontinued. It has been suggested by Sadun (personal communication) that the cause of the poor definition in the tests was the aging of the antigen.

The skin tests provide an indirect means for detecting antigen-antibody interaction through the resulting host tissue response. Since these tests depend on tissue response they possibly give an indication of the relationship between detectable antibodies and resistance to infection.

The first reference to an intradermal test, giving a rapid result when an antigen was injected intradermally came from Fairley and Williams (1927) who used an antigen derived from crushed livers of infected snails. Alves and Blair (1946) used a phenolic extract of cercariae dried on filter paper, and they found that it gave satisfactory results when used as a negative screening test. They, and many subsequent workers, considered that the test might be used as a criterion of cure. However, in the present investigation there were a number of apparently false positive reactions which indicate , as others have suggested, that individuals remain positive to the skin test for a considerable period after apparent cure.

Antigens for use in intradermal tests have been prepared from almost every stage in the life cycle of the parasite. There have been differences in results, particularly using whole worm or egg antigens, which suggest stage specificity of response, and Kagan (1958) suggests that this fact may be used as a criterion of cure.

In a series of comparative diagnostic trials undertaken in two communities in Rhodesia, a total of 519 people were tested by intradermal tests, using the World Health Organisation reference skin test antigen, and by examination of urine and stool specimens. 261 were found to be passing eggs, and of these known infected people 214 (82 percent) gave positive skin test response, but 47 (18 percent) failed to show a response. The majority of these failures of the skin test occurred in young children, and it is believed that there is a tendency for the skin test to fail to detect very new infections. Of the 258 people who were not found to be passing eggs, 152 (58 percent) gave positive reactions; as with the fluorescent antibody test for bilharziasis the data for skin tests for bilharziasis illustrates that a higher proportion of apparently false positive reactions occur in older people. These data are discussed later in this section and it is believed that this is an effect of the development of resistance to the disease.

Numerous other immunological tests have been evolved for detecting antibodies of a schistosome infection. These tests are essentially only minor modifications of the tried and proven diagnostic procedures used for studies on virus and bacterial infections. These procedures are used only to demonstrate detectable antibodies, and they do not necessarily indicate the concurrent presence of protective antibodies. However, the formation of precipitates round schistosomulae when they are incubated in immune serum is possibly an indication of the presence of circulating antibodies which afford a protection to the host against invading parasites. Newsome and Robinson (1956) incubated schistosomulae at 37°C. for 48 hours in immune serum. They observed that precipitates were formed at the oral sucker and at the excretory pore of schistosomulae which had been allowed to develop for 14 to 21 days from the time of invasion of the host. Robinson (1956) demonstrated that this precipitate developed only with young worms - of up to three weeks development; after this age, no precipitates were formed. Newsome and Robinson (op. cit.) concluded that the formation of these precipitates was partly responsible for the great reduction of invading parasites which occur in resistant hosts. The reduction has been shown by Olivier and Schneidermann (1953) and by Vogel and Minning (1953) to occur mainly in the lungs 10 to 14 days after invasion, and this is consistent with the age at which greatest precipitate formation occurs.

The formation of this precipitate on schistosomulae may be an indication of the protective nature of serum from resistant animals, and the fact that the stage at which this occurs corresponds to the stage at which highest mortality of parasites invading a resistant host occurs adds weight to this assumption.

Until the serum from resistant persons or animals can be clearly demonstrated to have a protective nature, it cannot be accepted that resistance depends on a circulating antibody. Sadun and Lin (1959) reported that, in passive serum transfer experiments, mice receiving serum from resistant rabbits developed a slight resistance, as indicated by a slight decrease in worm burden. This is not borne

out by the reports of other workers, including Vogel and Minning (op. cit.) and there is little direct evidence of the existence of circulating protective antibodies.

Whatever the mechanism of protection may be, there is undoubted evidence of immunological response. The presence of detectable antibodies is demonstrated by the serological and immunological tests, and there is no reason to doubt the parallel development of protective circulating antibodies.

Epidemiological Evidence of the Development of Resistance to Infections of Schistosoma spp. in man.

It has been stated that considerable evidence of the influence of acquired resistance to bilharziasis can be derived from both clinical observation and from epidemiological data.

Newsome (1956) asserts that clinical observation alone confirms the existence of some mechanism which increases tolerance of the worms or actively restrains the disease in older people. He discusses the work of Fujinami, who, in 1916, "remarked, what many have since described, that uninfected persons entering an endemic japonicum area become very ill, while the local inhabitants, at any rate the older ones, were less ill." Gelfand (personal communication) states that in Rhodesia, urinary bilharziasis, as a clinically manifested disease, is essentially a disease of children who appear to overcome the infection as they grow older. Honey and Gelfand (1960) reported that although it is a childhood disease, late sequelae, resulting from the tissure responses to earlier infections, appear from the second decade of life onwards, but the majority of these appear in the second or third decades. They reported that 70 percent of the cases of demonstrated gross calcification of the bladder occurred before the age of 30.

Many reports refer to decreased passage of S. haematobium eggs in older people and some relate this decrease to the development of resistance. Gerber (1952) studied the prevalence of the disease in different age groups of people in several villages in the Boadjibu

district of Sierra Leone. The people of the different villages were subjected in nature to different conditions of exposure to infection, and Gerber concluded that an immunological protection was evidenced. He stated that maximum protection was developed in people with a moderate initial exposure followed later by regular re-exposure to infection such as may be incurred by occasional exposure during one transmission season and followed by an exposure-free interval lasting to the next season. Pesigan et al. (1958) state that one factor in respect of the prevalence of S. japonicum in the Philippines, namely the downward trend with advancing age after the peak of prevalence is reached, could be explained as being partly due to host reaction, with an immunity mechanism. Several other workers, including Dixon (1934), Basseres and Pantoja (1947), Cowper (1953), and Maclean et al. (1958), report on the downward trend of prevalence after the age of peak prevalence, and most of these authors suggest a possible relationship with resistance. Morley-Smith and Gelfand (1960) examined over 1,000 people from an African farming district of Rhodesia, and they found a steady fall in prevalence from the peak which occurred in the first decade of life. After the age of 40, there was no further decline and the proportion of people infected remained steady thereafter at approximately 15 percent. They conclude that their studies support the existence of resistance acquired by the human body when the infection has been contracted some years previously.

The majority of these authors failed to consider that the decreased egg passage might have been due to decreased contact with infected water by the older people. However, under conditions existing in rural areas of Rhodesia, and particularly in the area in which Morley-Smith and Gelfand worked, it may be assumed that all people are regularly exposed to infection, since the only available water is invariably from a river or stream.

Manson-Bahr (1958) suggested that the development of resistance was dependent on the degree of transmission. He suggested that the decrease in the numbers of infections with advancing age, commonly

occurring in east and southern Africa, was the result of the development of immunity which increased with age. He also suggested that in areas of very intense transmission, such as occur in Egypt, the repeated heavy infections of very young children may so weaken the children that the development of immunity is hindered. This, he states, alters the clinical picture of the disease in such areas.

Surveys in Rhodesia.

Results obtained from extensive surveys to assess the prevalence of both S. capense and S. mansoni in African communities in Rhodesia have revealed further and more detailed evidence of the development of resistance, and of the conditions of exposure and infection which give rise to increased protection. These surveys have been conducted in communities in different areas of the country, and these areas were chosen to give a range of climatic, topographical and other conditions which in turn influence the level of infection to which the community is exposed. In most cases, an attempt was made to investigate stable and concentrated communities, which had similar standards of nutrition, housing and hygiene, and which differed from one another as little as possible, excepting for the climatic conditions, and the extent and nature of exposure to bilharziasis. In all the communities investigated, with the exception of the combined community of the Chikwanda and Zimutu, and of the Serima tribal trust areas, adequate and balanced food rations are provided to all people by their employers. Since these rations are all based on a recommended scale, the nutritional status of these communities is as uniform as is possible under African conditions. The recommended scale is given in appendix 2.

The following communities, the situations of which are shown on the map of Rhodesia (Fig. 8) were investigated.

1. The Chikwanda, Zimutu and Serima tribal trust areas, covering 40,000 hectares, lie in the catchment area of Lake Kyle, which supplies water to the Triangle and Hippo Valley sugar estates. They are separated by European owned farming areas, and they lie between 10 and

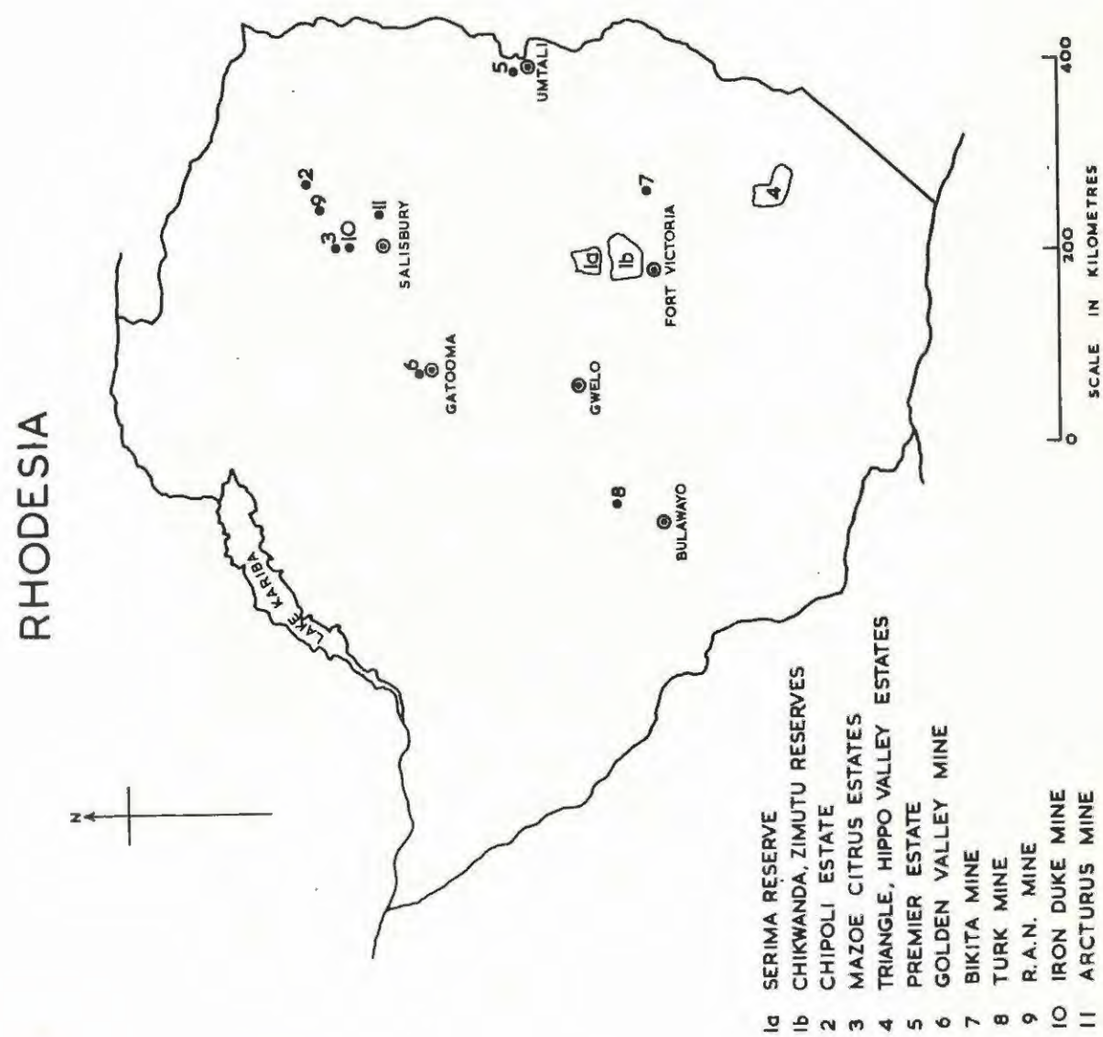


Figure 8. Map of Rhodesia showing main towns and situations of communities examined.

60 Km. north of Fort Victoria. For the purposes of this study, these three very similar areas are considered as having one large community. They are well watered areas but, because the soil is not fertile, they are not heavily populated. These were the only areas examined where the people do not receive the balanced diet listed in appendix 2. They live slightly above the subsistence level, deriving their livelihood mainly from the few cattle the area can support, and the poor crops of maize grown in the infertile soil.

The people are entirely dependent on natural surface water for all their needs, and latrines are absent, even from the schools; the standards of living and hygiene are therefore very low. Snails were common in all the waterways but the populations were not dense.

Transmission of bilharziasis was believed to be essentially seasonal and it probably occurred only in two short periods of the year, the first being from March to May, after the heavy rains but before the cold of the winter, and the second from October to December, during the hot summer before the onset of the rains. Snail control measures were instituted late in 1959. These measures were based on the application of chemical molluscicides to all the surface water in the area. The snail populations were drastically reduced after two such applications, and thereafter, the populations were kept to a very low level by the system of snail surveillance (Clarke et al. 1961). These control measures have been maintained up to the present time. They have proved most successful, snails only rarely having been found after April, 1960.

Surveys to assess the prevalence of S. capense infections in children were undertaken in 1960 and 1962, being conducted on both occasions in the months of February and March. Single urine specimens were examined from each child, and, although no attempt was made to examine specimens from the same children over successive years, the same villages were visited on each survey.

Climatic data: (Figures are given for the Serima area in the north, and, to represent the variations which are encountered, the data for Fort Victoria, lying a short distance to the south of the Chikwanda area, are also given.)

Situation: Serima township - latitude 19° 28'S., longitude 31° 03'E.
Fort Victoria - 19° 59'S., 30° 55'E.

Altitude above sea level: Serima township 1393 m.
Fort Victoria 1097 m.

Mean annual rainfall: Serima township 80 cm.
Fort Victoria 71 cm.

Temperature: Summer (October) mean maxima for month:
Serima township 27°C.
Fort Victoria 30°C.

Summer (October) mean minima for month:
Serima township 14°C.
Fort Victoria 15°C.

Winter (June) mean maxima for month:
Serima township 19°C.
Fort Victoria 21°C.

Winter (June) mean minima for month:
Serima township 7°C.
Fort Victoria 6°C.

Estimated total populations in 1959:
Chikwanda and Zimutu areas 32,500
Serima area 5,240

2. The Chipoli Irrigation Estate. This privately owned, mixed farming estate, established early in this century, is situated approximately 112 Km. north-east of Salisbury. The main irrigated crop is citrus, and all irrigation is of the flood type. There are two canal systems drawing water from the Mazoe River, the principal one having over 11 Km. of main canal, normally running at up to 1,000 cubic metres an hour, and having a total of 33 Km. of smaller distribution canals and furrows. The second has only 4 Km. of main canal, flowing at

500 cubic metres an hour, and there are no distribution canals or furrows. Most of the canals and all the furrows are unlined, and they support plant growth, particularly of Nitella spp. and Potamogeton sp.; they are normally cleared of this vegetation once a year, during the rains. The canals flow through two large dams, each impounding more than 50,000 cubic metres of water, and there are a further six dams on the estate impounding between 5,000 and 20,000 cubic metres each. All waters, including reservoirs, canals and furrows are heavily infested with snails, including Biomphalaria pfeifferi and Bulinus (Physopsis) globosus.

There is a labour force of 250, and the total African population is approximately 800. All domestic water is obtained from the canals, and the reservoirs and canals are also extensively used for swimming and fishing. Although latrines are supplied they are seldom used, the people preferring to use the citrus groves or the surrounding uncultivated lands for defaecation: urination occurs almost anywhere, the people, particularly the males, showing little desire for privacy. Contamination of the water with urine and faeces is therefore frequent.

Chipoli lies in the hot lower Mazoe Valley and, with the climatic conditions, the abundance of surface water, the extensive snail colonies, and the continuous and intimate contact the people have with the water, it would be expected that the level of transmission would be exceptionally high during the summer, and it would remain high throughout the year.

A stool and urine specimen examination survey to assess the prevalence of bilharziasis was undertaken in February, 1964.

Climatic data:

Situation: latitude 17° 14' S., longitude 31° 40' E.

Height above sea level: 968 m.

Mean annual rainfall: 80 cm.

Temperatures:	Summer (October) mean maxima for month	32°C.
	Summer (October) mean minima for month	16°C.
	Winter (June) mean maxima for month	26°C.
	Winter (June) mean minima for month	7°C.

3. The Mazoe Citrus Estates extend in a narrow strip for a distance of 20 Km. along the banks of the Mazoe River. These very large estates draw their water from the Mazoe Dam which impounds 35 million cubic metres of water. Considerably more than 1,000 people live on the estates. All the irrigation is of the flood type, and the water is brought to the lands by means of three separate canal systems, the largest carrying up to 2,000 cubic metres an hour, the other two each taking approximately 1,000 cubic metres an hour. The main canals are mostly lined with asbestos-cement corrugated sheeting, although some sections remain unlined. The smaller distribution canals are all concrete lined, and they only carry water on a cycle of one day in two weeks.

There is a concrete swimming bath for the children of the labourers and piped water is supplied to all housing compounds. However, since this water, and the water used to fill the swimming bath, is drawn straight from the canals without any form of purification, it must be accepted that the people are without safe water for either domestic use or for recreation.

Silt tends to be deposited in the troughs of the corrugated asbestos-cement lining of the canals, and this silt supports plant growth, which is permanent since these canals are never permitted to stop flowing. Snails of the species B. pfeifferi and B. (P.) globosus are therefore plentiful throughout the canal systems: B. pfeifferi was found in large numbers in the concrete swimming bath. No attempt has yet been made to control the snail populations in this irrigation system. Transmission of both forms of the disease was expected to be high and perennial.

A survey to assess the prevalence of bilharziasis was undertaken in June, 1963. This survey included stool and urine examinations, fluorescent antibody tests, intradermal tests, and the plasma card flocculation tests.

Climatic data:

Situation: (at meteorological station)

latitude 17° 28'S., longitude 31° 01'E.

Height above sea level: 1234 m.

Mean annual rainfall: 93 cm.

Temperatures: Summer (October) mean maxima for month 31°C.

Summer (October) mean minima for month 13°C.

Winter (June) mean maxima for month 23°C.

Winter (June) mean minima for month 5°C.

4. The Triangle and Hippo Valley sugar estates are two contiguous irrigated estates situated in the very hot, low-lying, arid and flat southern part of Rhodesia, the Triangle township being 130 Km. south-east of Fort Victoria. The estates at present have 20,000 hectares under irrigation, which is partly of the flood type, and partly under overhead spray. It is planned to extend the irrigation in this part of Rhodesia to 50,000 hectares by the end of 1965, and to 300,000 hectares within the following seven years. At present the water for irrigation is brought from the large Kyle lake, near Fort Victoria, and from the Bangala dam on the Mtilikwe River.

The total population is at present over 11,000 people but new-comers are swelling this figure by over 2,000 each month; it has been estimated that, within ten years, the production of sugar alone will support over one million people in the area.

The extensive canal systems, with large numbers of small night storage reservoirs, the unprotected drains leading away the regenerated water, the lack of sanitation or safe water supplies, the high temperatures throughout the year, and the concentration of people, make it certain that, unless some form of prevention is initiated, transmission of both forms of bilharziasis will be maintained at a very high level throughout the year. At present, the prevalence is not exceptionally high, because the large majority of the people tested are new to the area.

A stool and urine examination survey was conducted during February, 1964.

Climatic data:

Situation: the area is bounded by latitudes 20° 50'S. and 21° 10'S., and longitudes 31° 20'E. and 31° 50'E.

Height above sea level: 421 m.

Mean annual rainfall: 60 cm.

Temperatures: Summer (October) mean maxima for month 34°C.
Summer (October) mean minima for month 17°C.
Winter (June) mean maxima for month 26°C.
Winter (June) mean minima for month 7°C.

5. The Premier Citrus Estate, 30 Km. north-west of Umtali near the eastern border of Rhodesia, was brought under flood irrigation in 1898. Approximately 600 people reside on the estate. The water for the irrigation is brought from the Odzani River by means of an unlined canal, 14 Km. long, which runs through three earthen night storage reservoirs. The houses for the labourers and their families are sited near the reservoirs and canals, which form the sole source of domestic water. Latrines are provided, but they are not always used. Although snails of both important species were found, they were not as plentiful as expected; no reason could be found to explain this fact. It was expected that incidence would be moderately high, and that transmission would be seasonal.

Climatic data:

Situation: latitude 18° 55'S., longitude 32° 33'E.

Height above sea level: 1018 m.

Mean annual rainfall: 80 cm.

Temperatures: Summer (October) mean maxima for month 30°C.
Summer (October) mean minima for month 16°C.
Winter (June) mean maxima for month 23°C.
Winter (June) mean minima for month 7°C.

6. The Golden Valley Mine (gold) lies 20 Km. north-west of Gatooma in the central part of Rhodesia. It is in a flat, dry, hot area and there is little surface water in the neighbourhood. Over 700 people live on the mine, and they are provided with piped domestic water from underground supplies. There is a temporary stream 3 Km. from the mine, but since this strean is dry for several months each year, it is not considered to be an important source of infection. As with the majority of other communities investigated, the people living on this mine are well fed and well housed, and the standards of living and hygiene are relatively high.

It was expected that the incidence of bilharziasis would be low, and that transmission would be seasonal. A survey for bilharziasis was undertaken in December, 1963, and it included stool and urine examinations, intradermal tests and fluorescent antibody tests.

Climatic data:

Situation: latitude 18° 14'S., longitude 29° 48'E.

Height above sea level: 1157 m.

Mean annual rainfall: 84 cm.

Temperatures: Summer (October) mean maxima for month 32°C.
Summer (October) mean minima for month 17°C.
Winter (June) mean maxima for month 23°C.
Winter (June) mean minima for month 9°C.

7. The Bikita Minerals Mine (lithium) lies 75 Km. east of Fort Victoria, in an area with a large number of rivers and small streams. Over 200 people are employed on the mine and, with their families, there is a total population of over 600 people. The housing is well organised, and the houses are provided with piped water from underground sources. There are extensive recreational facilities, which tend to draw the people away from their normal pastimes of swimming and fishing in the surrounding rivers and streams. It was expected that transmission would be seasonal, and even then at only a moderately high level. A stool and urine examination survey for bilharziasis was undertaken

during March, 1964.

Climatic data:

Situation: latitude 20° 02'S., longitude 31° 30'E.

Height above sea level: 1100 m.

Mean annual rainfall: 80 cm.

Temperatures: Summer (October) mean maxima for month 30°C.
Summer (October) mean minima for month 15°C.
Winter (June) mean maxima for month 21°C.
Winter (June) mean minima for month 6°C.

8. The Turk Mine (gold) is in the western region of the country, 60 Km. north-east of Bulawayo. The district is very dry, and there is no permanent surface water in the vicinity of the mine except for a large dam, impounding over 50,000 cubic metres of water, 6 Km. distant. There is a large resident African community, and the people are well fed and housed, and they are provided with safe piped domestic water. There are extensive recreational facilities. The people have no need to visit the natural water for either washing, fishing or recreation, and therefore contact with infected water is limited. A stool and urine survey for bilharziasis was conducted in April 1964.

Climatic data:

Situation: latitude 19° 44'S., longitude 28° 47'E.

Height above sea level: 1219 m.

Mean annual rainfall: 60 cm.

Temperatures: Summer (October) mean maxima for month 31°C.
Summer (October) mean minima for month 16°C.
Winter (June) mean maxima for month 22°C.
Winter (June) mean minima for month 8°C.

9. The R.A.N. Gold Mine lies on the eastern boundary of Bindura township, approximately 55 Km. north-east of Salisbury. Over 120 men are employed on the mine, and the total population is over 500 people. The houses of the labourers are provided with piped water supplies from

underground sources, and latrines are also provided. There is a permanent water reservoir within two kilometres of the mine, and it is used by the people for washing and swimming. There is also one seasonal stream which crosses one of the most used roads to the mine. There are snails in both the stream and the reservoir. The mine lies in the central part of the hot Mazoe Valley, and it was expected that transmission would be perennial, with intense transmission occurring during the summer. However, the people of the mine have limited contact with natural water, except for recreational purposes, and the incidence of infection was expected to be high without being exceptional. A survey which included stool and urine examinations, and intradermal and fluorescent antibody tests was conducted during May, 1964.

Climatic data:

Situation: latitude 17° 19'S., longitude 31° 20'E.

Height above sea level: 1091 m.

Mean annual rainfall: 90 cm.

Temperatures: Summer (October) mean maxima for month 31°C.
Summer (October) mean minima for month 14°C.
Winter (June) mean maxima for month 23°C.
Winter (June) mean minima for month 6°C.

10. The Iron Duke Mine (iron pyrites) is situated 50 Km. north of Salisbury in the southern part of the Mazoe Valley. It lies adjacent to the perennially flowing Yellow Jacket River, a short distance from the point where this river flows through the Mazoe irrigated citrus estates. The housing for the mine, which has 600 resident Africans, is provided with piped water for all domestic requirements, but the proximity of the river and of the canals of the irrigation estates made it appear likely that the incidence of both S. mansoni and S. capense would be high. Because of the permanency of the waters and the relatively high temperatures throughout the year, it was expected that transmission would be maintained throughout the year,

with peak transmission occurring during the summer. The Yellow Jacket River was found to support very heavy populations of B. (P.) globosus and the canals of the irrigation estates were known to be infested with B. pfeifferi and B. (P.) globosus.

A urine examination survey to assess the prevalence of S. capense infections was conducted in February, 1964, and this was followed in August, 1964, by a stool examination survey to assess the prevalence of S. mansoni infections.

Climatic data:

Situation: latitude 17° 27'S., longitude 31° 04'E.

Height above sea level: 1230 m.

Mean annual rainfall: 93 cm.

Temperatures: Summer (October) mean maxima for month 31°C.
Summer (October) mean minima for month 13°C.
Winter (June) mean maxima for month 23°C.
Winter (June) mean minima for month 5°C.

11. The Arcturus Gold Mine, 25 Km. east of Salisbury, employs over 200 people, and there is a resident population of 800. The houses are all provided with piped water from an underground source, but the nearby streams and dams provide the people, and particularly the older children, with the opportunities for swimming, washing and fishing. It was expected therefore that the incidence of both S. mansoni and S. capense would be high, but that the transmission would be seasonal. Stool and urine surveys for bilharziasis were undertaken in July, 1964.

Climatic data: (except for the locality of the mine, all the data refers to Salisbury, since there is no meteorological data available from Arcturus. The two places are similar in climate.)

Situation: latitude 17° 47'S., longitude 31° 19'E.

Height above sea level: 1507 m.

Mean annual rainfall: 90 cm.

Temperatures: Summer (October) mean maxima for month 29°C.
Summer (October) mean minima for month 15°C.
Winter (June) mean maxima for month 21°C.
Winter (June) mean minima for month 7°C.

The results of these surveys have been analysed in respect of the ages of the people tested, and the results are presented in Tables 12 and 13. Where people of all ages were examined, they have been separated into eight age groups, selected to demonstrate the patterns of changes in prevalence of egg production with increasing age. These groups were also selected because it was normally possible to obtain population samples of sufficient size for each group, and because estimation of age between such groupings was simplified. In some of the communities examined, it proved difficult to persuade people between the ages of 13 and 20 to volunteer for testing. It appeared that people of these ages were either too embarrassed to submit specimens, or they considred it lacking in dignity to do so. This was unfortunate since this was one of the most interesting age groups. The older people were more tolerant and they readily volunteered for testing, especially if they knew that they would be informed of the results, and infected persons could obtain treatment. The results of these surveys are discussed in four sections as follows:

1. The Age-Prevalence Relationship and the Intensity of Infections.

The relationship of prevalence of bilharziasis to age of infected persons, where the prevalence is assessed by stool and urine specimen examinations, has been investigated in these surveys and the results add evidence of the development of resistance to S. capense infections in all the communities, and to S. mansoni infections in the communities of the Chipoli Estate, the Triangle and Hippo Valley sugar estates, and the Iron Duke and Arcturus mines (Tables 12 A-G and 13 A, B, F and G, and Figs. 9 A-D and 10).

In these communities there is a consistent pattern in the age-prevalence relationship. Even below the age of 4, some of the children in most communities show infection. In older age groups the numbers of children showing active infections increase to higher and higher proportions with increasing age, until a peak is reached, normally between the ages of 7 and 15. After the age of peak prevalence, the proportion of people passing eggs decreases with age until,

TABLE 12.

S. capense INFECTIONS: Results of Stool and Urine Examination Surveys in Seven Communities in Rhodesia.

A. CHIPOLI ESTATE:

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Total
MALES:									
Number examined	3	43	30	5	9	8	36	15	149
Number passing S. capense eggs	3	42	29	4	8	7	18	4	115
Percent passing S. capense eggs	-	98	97	80	89	88	50	27	77
FEMALES:									
Number examined	6	33	25	20	4	13	65	20	186
Number passing S. capense eggs	5	31	25	20	4	12	55	16	168
Percent passing S. capense eggs	83	94	100	100	-	92	85	80	90
TOTALS:									
Number examined	9	76	55	25	13	21	101	35	335
Number passing S. capense eggs	8	73	54	24	12	19	73	20	283
Percent passing S. capense eggs	89	96	98	96	92	90	72	57	84

B. TRIANGLE AND HIPPO VALLEY SUGAR ESTATES:

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Total
MALES:									
Number examined	15	47	41	27	35	81	97	73	416
Number passing S. capense eggs	6	17	27	21	23	46	30	3	173
Percent passing S. capense eggs	40	36	66	78	66	57	31	4	42
FEMALES:									
Number examined	15	40	25	11	7	25	140	43	306
Number passing S. capense eggs	3	9	12	7	5	12	49	5	102
Percent passing S. capense eggs	20	23	48	64	71	48	35	12	33
TOTALS:									
Number examined	30	87	66	38	42	106	237	116	722
Number passing S. capense eggs	9	26	39	28	28	58	79	8	275
Percent passing S. capense eggs	30	30	59	74	67	55	33	7	38

TABLE 12 (contd.)

S. capense INFECTIONS: Results of Stool and Urine Examination Surveys in Seven Communities in Rhodesia.

C. PREMIER CITRUS ESTATES:

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Total
MALES:									
Number examined	10	16	39	34	8	14	89	57	267
Number passing S. capense eggs	0	4	15	22	4	4	15	9	73
Per cent passing S. capense eggs	0	25	38	65	50	29	17	16	27
FEMALES:									
Number examined	15	21	38	20	11	14	59	13	191
Number passing S. capense eggs	0	6	22	10	5	6	8	1	58
Per cent passing S. capense eggs	0	29	58	50	45	43	14	8	30
TOTALS:									
Number examined	25	37	77	54	19	28	148	70	458
Number passing S. capense eggs	0	10	37	32	9	10	23	10	131
Per cent passing S. capense eggs	0	27	48	59	47	36	16	14	29

D. BIKITA MINERALS MINE:

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Total
MALES:									
Number examined	13	29	33	63	54	53	16	16	277
Number passing S. capense eggs	1	5	13	38	29	23	2	2	113
Per cent passing S. capense eggs	8	17	39	60	54	43	13	13	41
FEMALES:									
Number examined	5	11	33	43	23	25	6	4	150
Number passing S. capense eggs	0	3	9	22	12	11	2	0	59
Per cent passing S. capense eggs	-	27	27	51	52	44	33	-	39
TOTALS:									
Number examined	18	40	66	106	77	78	22	20	427
Number passing S. capense eggs	1	8	22	60	41	34	4	2	172
Per cent passing S. capense eggs	6	20	33	57	53	44	18	10	40

S. capense INFECTIONS: Results of Stool and Urine Examination Surveys in Seven Communities in Rhodesia.

E. TURK MINE:

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Total
MALES:									
Number examined	8	13	51	45	8	7	57	81	270
Number passing S. capense eggs	0	1	7	7	5	3	8	1	32
Per cent passing S. capense eggs	0	8	14	16	63	43	14	1	12
FEMALES:									
Number examined	10	12	49	31	5	8	57	21	193
Number passing S. capense eggs	0	2	6	3	0	1	7	0	19
Per cent passing S. capense eggs	0	17	12	10	-	13	12	0	10
TOTALS:									
Number examined	18	25	100	76	13	15	114	102	463
Number passing S. capense eggs	0	3	13	10	5	4	15	1	51
Per cent passing S. capense eggs	0	12	13	13	39	27	13	1	11

F. IRON DUKE MINE:

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Total
MALES:									
Number examined	14	34	34	18	17	2	17	22	158
Number passing S. capense eggs	1	19	26	15	16	2	4	1	84
Per cent passing S. capense eggs	7	56	76	83	94	-	24	5	53
FEMALES:									
Number examined	11	15	24	28	9	13	51	19	170
Number passing S. capense eggs	3	11	19	22	7	10	12	1	85
Per cent passing S. capense eggs	27	73	79	79	78	77	24	5	50
TOTALS:									
Number examined	25	49	58	46	26	15	68	41	328
Number passing S. capense eggs	4	30	45	37	23	12	16	2	169
Per cent passing S. capense eggs	16	61	78	80	88	80	24	5	52

TABLE 12 (contd.)

S. capense INFECTIONS: Results of Stool and Urine Examination Surveys in Seven Communities in Rhodesia.

G. ARCTURUS MINE:

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Totals
MALES:									
Number examined	17	32	36	35	37	10	55	27	249
Number passing S. capense eggs	1	5	11	24	28	4	13	2	88
Per cent passing S. capense eggs	6	16	31	69	76	40	24	7	35
FEMALES:									
Number examined	15	30	38	19	15	23	50	25	215
Number passing S. capense eggs	0	5	14	10	8	13	17	2	69
Per cent passing S. capense eggs	0	17	37	53	53	57	34	8	32
TOTALS:									
Number examined	32	62	74	54	52	33	105	52	464
Number passing S. capense eggs	1	10	25	34	36	17	30	4	157
Per cent passing S. capense eggs	3	16	34	63	69	52	29	8	34

TABLE 13.

S. mansoni INFECTIONS: Results of Stool and Urine Examination Surveys in Seven Communities in Rhodesia.

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Totals
A. CHIPOLI ESTATE:									
Number examined, males and females	8	70	51	23	15	24	95	32	318
Number passing S. mansoni eggs	4	58	47	20	10	12	56	16	223
Per cent passing S. mansoni eggs	50	83	92	87	67	50	59	50	70
B. TRIANGLE AND HIPPO VALLEY SUGAR ESTATES:									
Number examined, males and females	30	87	66	38	42	106	237	116	722
Number passing S. mansoni eggs	4	10	11	19	10	17	24	8	103
Per cent passing S. mansoni eggs	13	11	17	50	24	16	10	7	14
C. PREMIER CITRUS ESTATES:									
Number examined, males and females	21	36	73	56	15	28	148	68	445
Number passing S. mansoni eggs	1	6	9	19	3	2	32	8	80
Per cent passing S. mansoni eggs	5	17	12	34	20	7	22	12	18
D. BIKITA MINERALS MINE:									
Number examined, males and females	20	37	60	84	67	59	21	20	368
Number passing S. mansoni eggs	1	1	3	5	2	7	3	1	23
Per cent passing S. mansoni eggs	5	3	5	6	3	12	14	5	6
E. TURK MINE:									
Number examined, males and females	14	26	100	75	14	9	95	95	428
Number passing S. mansoni eggs	0	0	5	2	0	0	6	6	19
Per cent passing S. mansoni eggs	0	0	5	3	0	0	6	6	4
F. IRON DUKE MINE:									
Number examined, males and females	6	27	45	50	24	7	29	11	199
Number passing S. mansoni eggs	2	11	35	29	14	7	11	2	111
Per cent passing S. mansoni eggs	-	41	78	58	58	-	38	18	56
G. ARCTURUS MINE:									
Number examined, males and females	33	61	73	53	52	33	106	51	462
Number passing S. mansoni eggs	1	4	7	23	23	6	16	10	90
Per cent passing S. mansoni eggs	3	7	10	43	44	18	15	20	19

Figure 9. Prevalence of S. capense infections in relation to age and sex in four communities of Rhodesia.

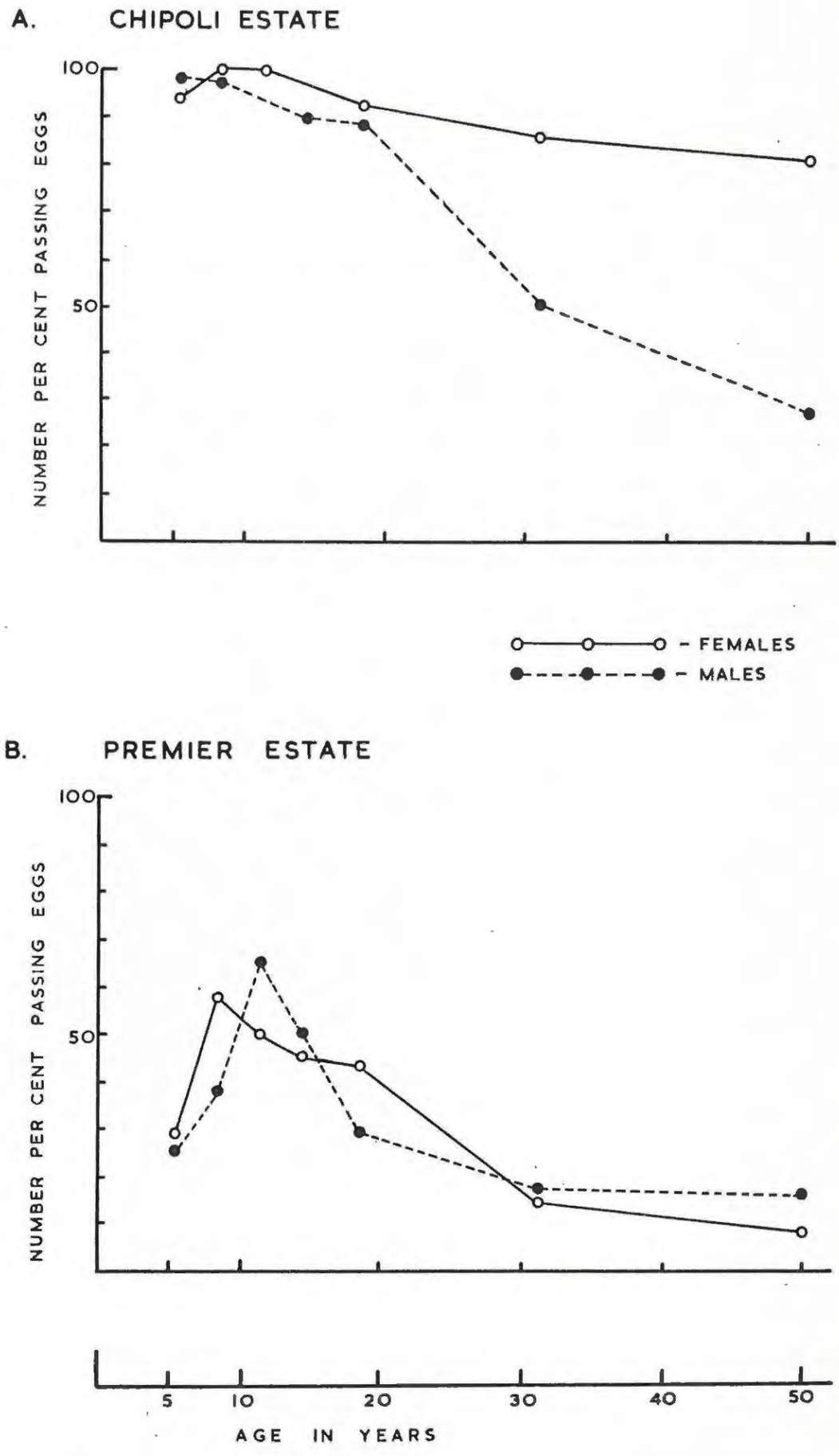
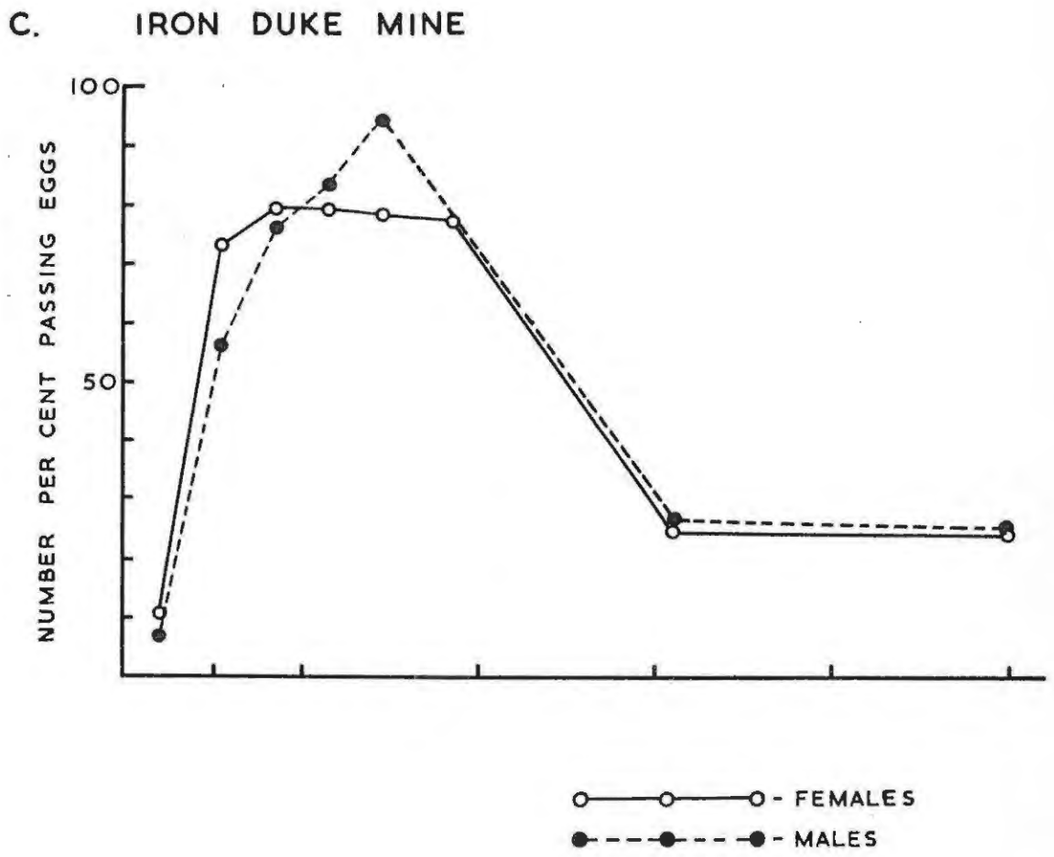
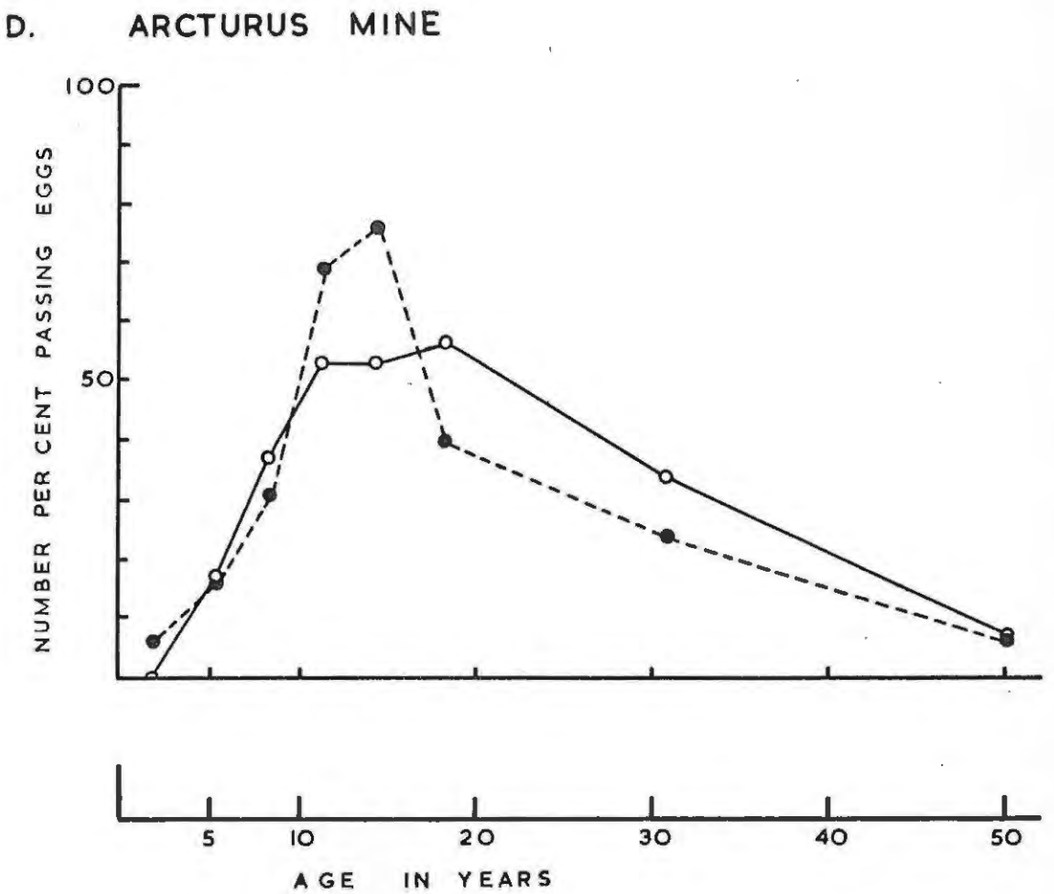


Figure 9 (contd.) Prevalence of S. capense infections in relation to age and sex in four communities of Rhodesia.





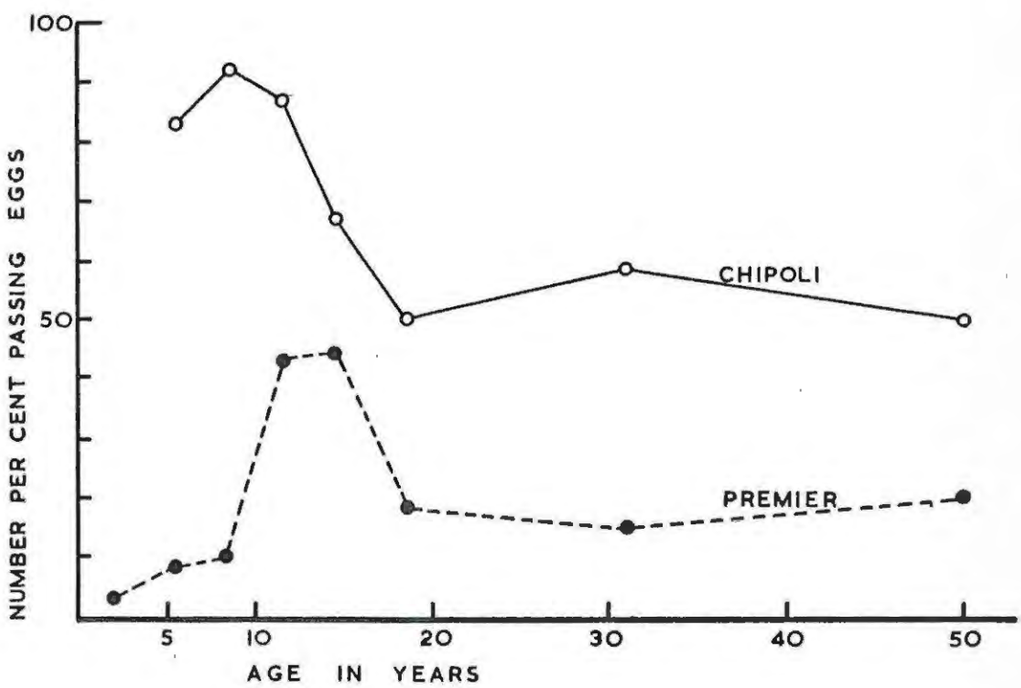


Figure 10. Prevalence of S. mansoni infections in relation to age in two communities in Rhodesia.

Erratum. For Premier read Arcturus.

in the older adults, the proportion of people showing active infections is usually very low. For example, in the males of the community of the Iron Duke mine, the peak of prevalence of S. capense infections is 94 percent, and this occurs in the age group 13 to 15 years. Subsequently, the prevalence decreases with age until only 5 percent of the males over 40 years old are still passing eggs. Similarly, on the Triangle and Hippo Valley sugar estates, the peak of prevalence of S. mansoni infection is 50 percent and this occurs in the age group 10 to 12 years; thereafter, the prevalence decreases with age to 24 percent in the age group 13 to 15, to 16 percent in the age group 16 to 20, to 10 percent in the age group 21 to 40, and finally to as low as 7 percent in the older adults over 40 years old.

These results confirm a picture of the prevalence rising with age until a peak is reached, and thereafter decreasing, sometimes dramatically with advancing age, until in older adults a relatively low level of detectable infection is reached, after which no further decrease takes place.

The age at which peak prevalence is reached varies considerably and, generally, it appears to occur earlier in life in areas of high incidence, and later in life in areas of low incidence. In some areas, where the level of transmission is very high, the peak is reached in the first decade of life. An example of this is the prevalence of S. capense infections on the Chipoli Estate where 97 percent of the boys aged 7 to 9 years showed infections. All of the 49 girls between the ages of 7 and 12 were passing eggs. Where the level of transmission is low, the peak of prevalence is reached later in life. This is seen in the community of the Turk Mine where the age groups 13 to 15 years and 16 to 20 years showed the highest prevalence of S. capense. The prevalence even in these two age groups was low when compared with that of the Chipoli Estate. Similar differences can be seen in the prevalence of S. mansoni infections in the communities of the Iron Duke and Bikita Mines.

At the Iron Duke mine where the prevalence is high, the peak of prevalence of 78 percent is reached in the age group 7 to 9 years, but in the Bikita mine community the peak of infection of 12 to 14 percent is reached in the age group 16 to 20 years and the young adults of 21 to 40 years.

At present there exists no satisfactory method for assessing the numbers of worms living or active in an individual, nor for assessing the average worm burden in a community or a section of a community. However, the numbers of S. capense eggs passed in the urine of an individual can be accepted as an indication of the activity or intensity of the infection. In urine examination surveys for S. capense infections in the communities of the Chipoli and Premier Estates and of the Bikita, Turk, Iron Duke and Arcturus mines, the eggs present in the urine samples were counted and the results for the different age groups were examined in terms of the average egg output in the infected members of the community. The results are presented in Table 14 and Fig. 11. The numbers of S. mansoni eggs in stool specimens at Chipoli Estate and the Bikita mine were also counted. The results are presented in Table 15. Counts of S. mansoni eggs in stool specimens were only made at Chipoli and Bikita. Egg counting in stool specimens is unsatisfactory since it is at present impossible to obtain reproducible results. The results in these two areas are only included because they indicate a tendency for decrease in numbers of eggs passed in older age groups.

The significance of the differences in the average numbers of eggs passed was established by the Kruskal-Wallis one way analysis of variance method (Seigal, 1956). The statistical analyses are given in Appendix 1 (page 146).

It can be seen from the tables and figures that, with the exception of the S. mansoni egg counts for the Bikita mine community and S. capense at the Turk mine, there is a consistent pattern of egg production in all the areas examined. In the other areas, the average egg count for the people passing eggs increased

TABLE 14.

S. capense INFECTIONS: Results of Stool and Urine Examination Surveys in Five Communities in Rhodesia.

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Totals
A. CHIPOLI ESTATE:									
Number examined, males and females	9	76	55	25	13	21	101	35	335
Number passing S. capense eggs	8	73	54	24	12	19	73	20	283
Total number of eggs	12240	187650	173460	85140	4670	12040	43230	3420	521850
Average number of eggs passed	1530	2571	3212	3548	389	634	592	171	1844
C. PREMIER CITRUS ESTATE:									
Number examined, males and females	25	37	77	54	19	28	148	70	458
Number passing S. capense eggs	0	10	37	32	9	10	23	10	131
Total number of eggs	0	5950	65710	28670	2860	940	3070	880	108080
Average number of eggs passed	0	595	1776	896	318	94	133	88	825
D. BIKITA MINERALS MINE:									
Number examined, males and females	18	40	66	106	77	78	22	20	427
Number passing S. capense eggs	1	8	22	60	41	34	4	2	172
Total number of eggs	60	5620	35780	97760	10520	16950	1230	120	168040
Average number of eggs passed	60	703	1626	1629	257	499	308	60	977
F. IRON DUKE MINE:									
Number examined, males and females	25	49	58	46	26	15	68	41	328
Number passing S. capense eggs	4	30	45	37	23	12	16	2	169
Total number of eggs	1670	12780	39920	14400	10100	3500	1240	20	83630
Average number of eggs passed	418	426	887	389	439	292	78	10	495
G. ARCTURUS MINE:									
Number examined, males and females	32	62	74	54	52	33	105	52	464
Number passing S. capense eggs	1	10	25	34	36	17	30	4	157
Total number of eggs	20	2950	12650	20180	8230	9480	4520	230	58260
Average number of eggs passed	20	295	506	594	229	558	151	58	371

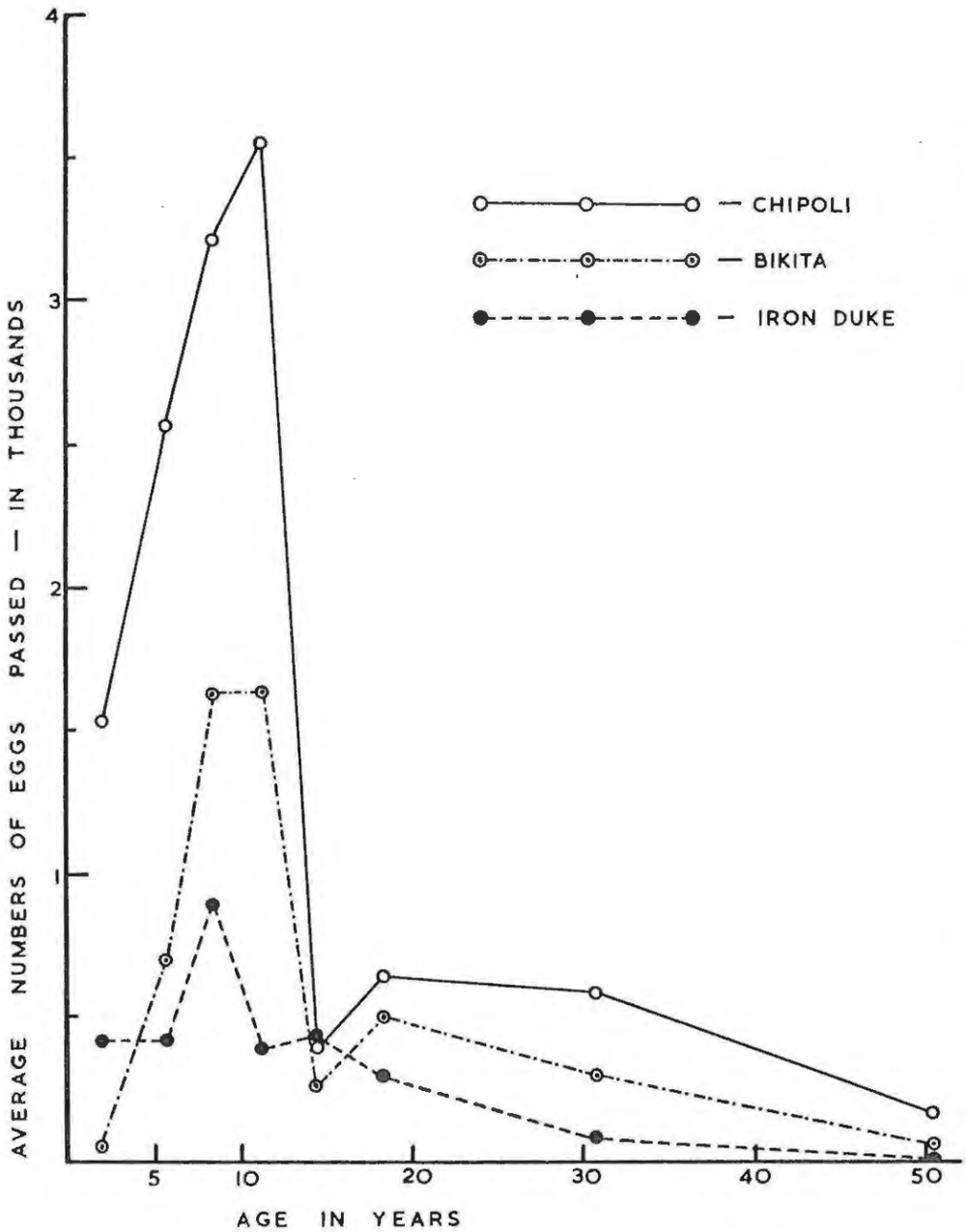


Figure 11. Average numbers of S. capense eggs passed by different age groups in three communities of Rhodesia.

TABLE 15.

S. mansoni INFECTIONS: Results of Stool and Urine Examination Surveys in Two Communities in Rhodesia.
Number of eggs passed.

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Totals
A. CHIPOLI ESTATE:									
Number examined, males and females	8	70	51	23	15	24	95	32	318
Number passing S. mansoni eggs	4	58	47	20	10	12	56	16	223
Total number of eggs	90	7620	3550	1380	266	750	2280	650	16586
Average number of eggs passed	23	131	76	69	26	63	41	41	74
D. BIKITA MINERALS MINE:									
Number examined, males and females	20	37	60	84	67	59	21	20	368
Number passing S. mansoni eggs	1	1	3	5	2	7	3	1	23
Total number of eggs	120	80	110	120	160	230	50	190	1060
Average number of eggs passed	120	80	37	24	80	33	17	190	46

rapidly to a peak, usually occurring between the ages of 7 and 12. Thereafter, the decrease in average egg production was dramatic within the two age groups succeeding the peak. There was often a second minor peak occurring between the ages 13 to 20 years, after which there was a further decrease in egg production with advancing age. This decrease in later life was slow but steady, without the dramatic decrease such as occurs immediately after the peak of egg production. No adequate explanation of this second minor peak can be given, but it is believed that it represents the more frequent contact with water by the young adults, particularly the young women, who, because of domestic duties, are so often compelled to have daily contact with surface water.

An examination of the proportions of infected people, in the different age groups, who pass large numbers of eggs reveals a similar pattern of younger people passing more eggs than the older people. Examples of this are given in Table 16 and Fig. 12 for S. capense infections. The actual numbers recorded for the Premier Estate are statistically analysed in Appendix 1 (page 150). This analysis demonstrates that the proportions of infected people passing large numbers of eggs differ in the age groups examined.

It can be seen that the proportion of children passing more than 200 eggs of S. capense in a one hour specimen, rises in early childhood and reaches a maximum for the community usually in those people between the ages of 5 and 10 years. There is a subsequent decline in this proportion and very few adults are found to be passing eggs in large numbers. The general trend of average egg production follows the same pattern as prevalence with advancing age; both prevalence and egg production rise to a peak in early life, and then decrease progressively with age. The age of peak prevalence usually occurs at approximately the same age as the peak of average egg production, or before it.

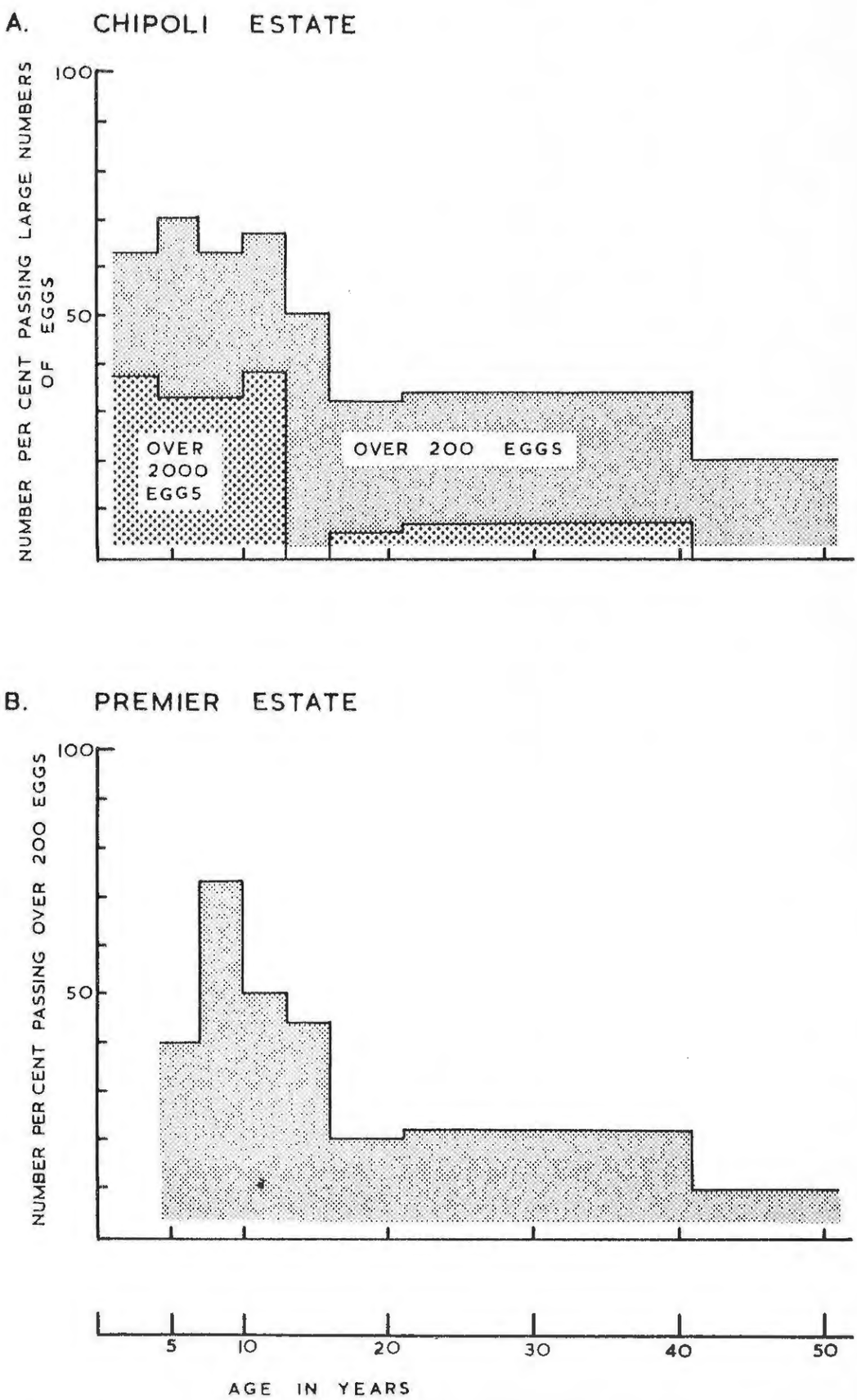
By comparing the proportion of heavily infected people in different areas, it can be seen that the severity of infection,

TABLE 16.

Proportion of infected persons passing largenumbers of S. capense eggs in six communities in Rhodesia.

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Total
Chipoli Estate:									
Number of people passing eggs	8	73	54	24	12	19	73	20	283
Number passing over 2001 eggs	3	24	18	9	0	1	5	0	60
Number per cent passing over 2001 eggs	37.5	33	33	38	0	5	7	0	21
Number passing over 201 eggs	5	51	34	16	6	6	25	4	147
Number per cent passing over 201 eggs	63	70	63	67	50	32	34	20	52
Premier Estate:									
Number of people passing eggs	0	10	37	32	9	10	23	10	131
Number passing over 201 eggs	0	4	27	16	4	2	5	1	59
Number per cent passing over 201 eggs	0	40	73	50	44	20	22	10	45
Bikita Mine:									
Number of people passing eggs	1	8	22	60	41	34	4	2	172
Number passing over 201 eggs	0	5	16	42	11	16	2	0	92
Number per cent passing over 201 eggs	0	63	73	70	27	47	50	0	53
Turk Mine:									
Number of people passing eggs	0	3	13	10	5	4	15	1	51
Number passing over 201 eggs	0	2	5	5	0	1	5	0	18
Number per cent passing over 201 eggs	0	67	38	50	0	25	33	0	35
Iron Duke Mine:									
Number of people passing eggs	4	30	45	37	23	12	16	2	169
Number passing over 201 eggs	2	12	26	11	10	5	1	0	67
Number per cent passing over 201 eggs	50	40	58	30	43	42	6	0	40
Arcturus Mine:									
Number of people passing eggs	1	10	25	34	36	17	30	4	157
Number passing over 201 eggs	0	4	12	17	10	6	6	0	55
Number per cent passing over 201 eggs	0	40	48	50	28	35	20	0	35

Figure 12. Proportions of infected people passing over 200 and over 2000 S. capense eggs in one hour in two communities of Rhodesia.



as judged by the egg production, is greatest where the prevalence of infection is high. This is in any case a reasonable supposition since it may be expected that, where a high level of transmission is maintained, not only will a larger number of people become infected, but they will be exposed to heavier infections. However, even in the most heavily infected area examined there is still a marked drop in egg production in older people.

The surveys show that in each community there is a relationship, at the time of the survey, between the prevalence of infections, the intensity of these infections, and the age structure of the population examined. This relationship was apparent as an increasing prevalence and intensity with age until a peak was reached, usually between the ages of 7 and 20 years. Thereafter, the prevalence and intensity of infections decreased with advancing age.

It is logical to assume that as the individuals pass from one age group to the next, the proportion of these individuals passing eggs and the numbers of eggs passed by the infected individuals will either increase or decrease in a similar manner. Although prevalence refers essentially to the number of proportion of people examined who are infected at the time of the survey, it can be assumed that the results reflect the changing pattern of prevalence and intensity in a group of individuals as they pass from the youngest age group through to the oldest.

On this assumption it seems that there is a dynamic pattern of prevalence with age. Few if any infections are found in the youngest children examined, except in areas of intense transmission. As they progress through the age groups, more and more of them become infected until a peak of prevalence is reached. After this peak, people appear to lose the infections and the prevalence decreases. There is a corresponding decrease in the numbers of eggs passed by those individuals who remain infected.

It might be argued that the children between the ages of 7 and 20 years are likely to have more contact with water than the older

people, and that the differences in the extent of exposure to infection may account for the decrease in prevalence observed in older people. This argument, which is discussed later in this section, cannot explain the striking decrease in prevalence and egg production which is seen in older people.

There are two possible explanations for the decrease in egg production with age. Firstly, that the deposition of fibrotic tissue resulting from the prolonged passage of eggs through the bladder or intestinal wall forms a mechanical barrier to further egg passage, or secondly, that the acquired resistance is developed by the host. In fact it is probable that both these factors influence the pattern of egg production in different age groups since it has been established that extensive fibrosis of the bladder wall can result ffom an infection, and it will limit or prevent egg passage. However, there are several reasons for rejecting the contention that fibrosis is the sole factor limiting the passage of eggs. These are:

(a) In the community of the Chipoli Estate where infections are at a very high level, and where it would be expected that fibrosis of the bladder wall would be severe, the egg production shows the least tendency to decrease of all the areas examined. This is entirely contrary to the expected if it is accepted that fibrosis alone limits egg passage.

(b) The decrease in egg passage in human cases is often accompanied by a relief in clinical symptoms. This would hardly be the case if, in fact, the activity of the worms was maintained and the eggs were trapped in the body. A much more pronounced pathogenesis would be expected to result if such was the case.

(c) In areas of high S. mansoni prevalence (Table 13 and Fig. 13) there is also diminished egg produc-

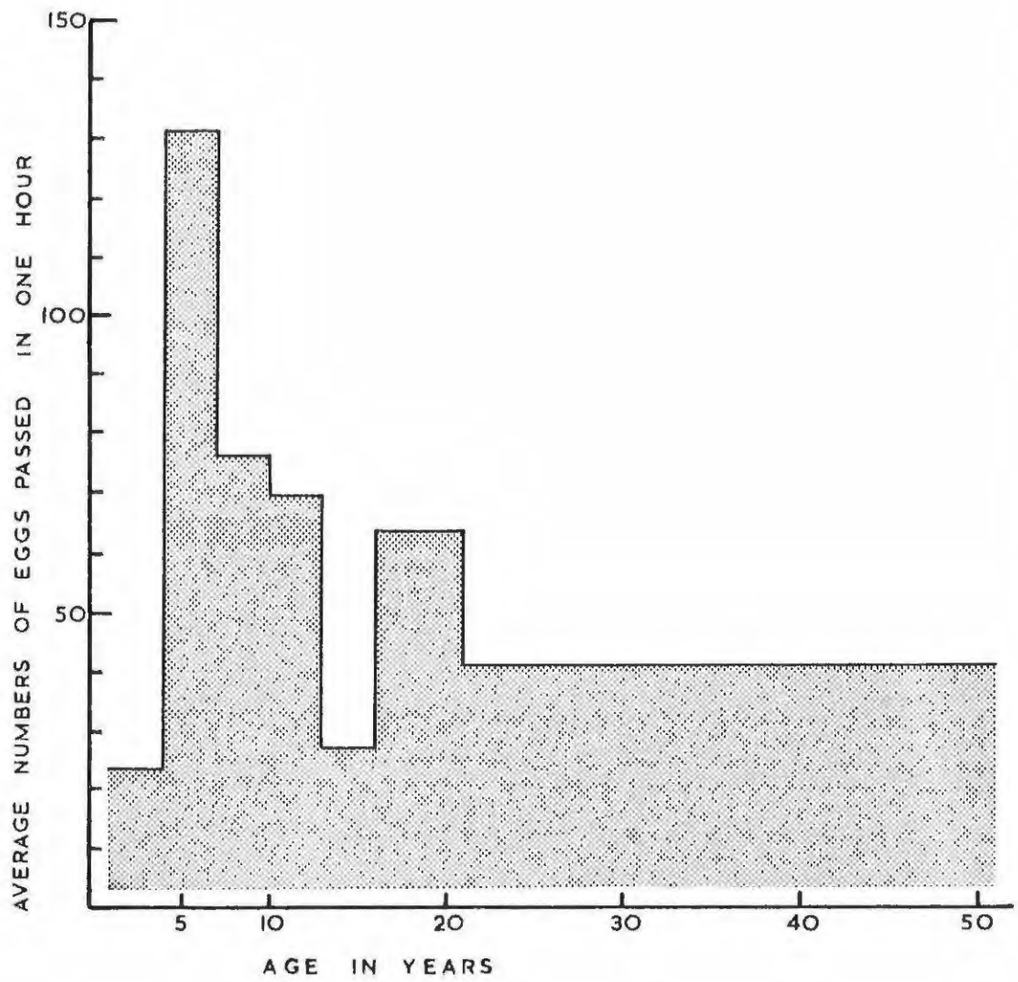


Figure 13. Average numbers of S. mansoni eggs passed in one hour by infected people of different age groups of the Chipoli Estate.

tion in adults. This cannot be explained as resulting from fibrosis of the intestinal wall.

(d) In animal experiments, with S. mansoni or S. japonicum infection in monkeys, the fall in egg production in individual monkeys has been repeatedly observed (Vogel and Minning 1953; Jachowski and Anderson, 1961) without any reported evidence of substantial fibrosis at autopsy. McMullen (personal communication) states that monkeys which had been exposed to very heavy infections of S. mansoni were examined after death. These monkeys had shown strong resistance to infection, yet no fibrosis could be found which could be attributed to the infection.

It must be concluded therefore that the older people have acquired a resistance to the infections and that this resistance influences the activity, and probably the life span, of the parasites, resulting in decrease in egg production in older people. This resistance is probably a host response, and it is therefore an immunological resistance.

It appears that there is a relationship between the development and the degree of resistance on the one hand, and the degree, nature and duration of infection, re-infection and superinfection on the other.

A theoretical interpretation of this relationship can be suggested as follows:

In the earliest years of exposure to infection the children are lacking in any resistance and infection takes place. Slightly older children are more frequently exposed to infection, and more of them become infected. At the same time, those that are infected develop partial resistance, and the longer they are infected the greater will be the degree of developing resistance. The early

resistance is weak and reinfections and superinfections occur. These tend to fortify the resistance, and they are themselves overcome more rapidly than the initial infections. After several years, the children infected in the early years of life have developed a more complete immunity and others, infected later, are showing the first partial resistance. Thus the resistance in the people in the community develops until, at the age of peak prevalence, there is a balance between the number of new infections or reinfections and the number of infections being overcome as a result of acquired resistance. After the peak of infections, the numbers of infections overcome because of the acquired resistance becomes progressively greater than the number of people showing reinfection, and the prevalence therefore decreases with age.

The variations in the degree of host immune response in individuals and the conditions and extent of re-exposure determine the rate of decrease in the observed prevalence. There is invariably a small proportion of adults who apparently either remain susceptible to reinfection, or who are unable to throw off the initial infection. In areas where perennial transmission is maintained at a very high level, it is apparent that the acquired resistance can be overwhelmed by exposure to heavy infection; it is in these areas that the decrease in observed prevalence will be less pronounced than it is in areas of moderate transmission. A possible example of this is seen in the results for S. capense infections in the community of the Chipoli Estate.

Many authors have commented on observed differences in egg prevalence relationships in the two sexes. It appears from the present studies, however, that these differences are not the results of differences of immune responses in the sexes, but rather to the different exposure patterns related to occupations and sociological habits of the sexes. This is particularly striking at Chipoli where the prevalence in adult women is higher than it is in adult men. This is because the only sources of domestic water are the heavily

infected canals and furrows. In the course of their domestic duties the women are compelled to have regular contact with the water of these canals, but the system of irrigation in practice on this estate does not require regular or prolonged contact with the water by the men.

The egg production patterns in relation to age, and the age-prevalence relationships have so far been considered separately. However, the full effects of resistance in a community can be best appreciated by examining both egg production and age prevalence together. Jordan (1963) referred to an "infection potential" for any age group. He derived this from the product of the average egg production and the prevalence, percent, in that age group. Jordan used this measure only to attempt to demonstrate that children were largely responsible for maintaining the infection of snails, and that control measures should be directed towards preventing infection in these children. However, this infection potential does demonstrate the dramatic decrease in egg production in older people, when this is considered in relation to the decrease in age prevalence which is observed in the same age groups. The infection potentials of the age groups examined at Chipoli and Premier Estates are given in Table 17, Fig. 14. This demonstrates that, even in the community of Chipoli Estate, there is a dramatic decrease in the numbers of eggs passed by the older people. Partial resistance must be influencing egg production even in this community.

Specificity of Resistance.

A further conclusion which can be drawn from the epidemiological data refers to the specificity of the acquired resistance.

The majority of workers in the field of immuno-diagnosis have demonstrated a cross antigenicity between the species of the genus Schistosoma, and other workers, including Anderson (1960) and Sadun (1963) have reported cross antigenicity between the genus Schistosoma and other genera including Trichinella, Heterophyes and Fasciola. However, Anderson et al. (1963) demonstrated genus

TABLE 17.

Infection Potentials in Age Groups at the Chipoli and Premier Estates.

	Age groups examined								
	Under 4	4 - 6	7 - 9	10 - 12	13 - 15	16 - 20	21 - 40	40 +	Total
Chipoli:									
Mean egg production	1530	2571	3212	3548	389	634	592	171	1844
Percent passing eggs	89	96	98	96	92	90	72	57	84
Infection potential	136,170	246,816	314776	340,608	35,788	57,060	42,624	9,747	154,896
Premier:									
Mean egg production	0	595	1776	896	318	94	133	88	825
Percent passing eggs	0	27	48	59	47	36	16	14	29
Infection potential	0	16,065	85,248	52,864	14,946	3,384	2,128	1,232	23,925

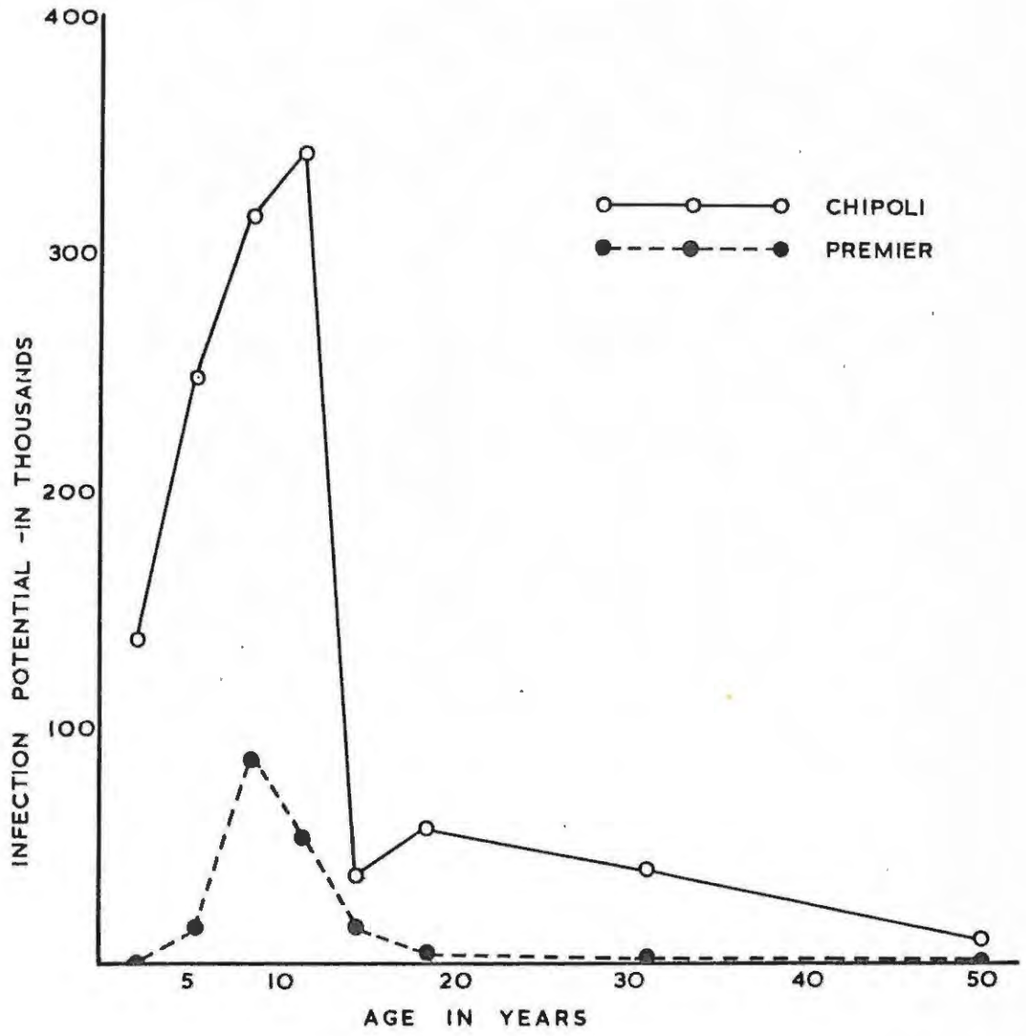


Figure 14. Infection potentials (Jordan, 1963) of different age groups in two communities of Rhodesia.

specificity of certain fractions of antigen extracts where the crude extracts gave cross reactions between related genera. Despite the evidence that antigen extracts contain a multiplicity of antigenic fractions, the intergeneric and the intrageneric nature of the antigen-antibody reactions in immuno-diagnosis has led to the belief that acquired resistance will follow a similar pattern of cross reaction between the species of Schistosoma, and that resistance acquired as a result of infection by one species will protect the host against infection by a second species.

No conclusive work has been reported on inter-species resistance in animal experiments, and, up to the present, there has been no information on the specificity of resistance in man.

The pattern of prevalence increasing to a peak, usually in children or young adults, and then decreasing with advancing age, has been observed for all the species of Schistosoma normally infecting man. Pesigan et al. (1958) showed this pattern for S. japonicum infections in the Philippines, where the peak of prevalence occurred in young adults. Basseres and Pantoja (1947) found the peak of prevalence of S. mansoni infections in Minas Gerais, Brazil, in the 15 to 24 year age group, and a reduced prevalence was observed in the older people. Gerber (1952) observed a similar pattern of S. haematobium infections in people in Sierra Leone. The present investigation in Rhodesia has shown that the prevalence of S. capense, and in some areas, of S. mansoni, reaches a peak and then decreases rapidly with advancing age. Thus it may be accepted that all species normally infecting man are capable of stimulating resistance.

If resistance to one species influences the incidence and prevalence of another species, it would be expected that the age-prevalence relationship would show a similar pattern for both species in an area where both species are endemic. However, it has been found that in an area where the incidence of S. capense is high, but that of S. mansoni is low, the pattern of the age-prevalence for each species is such as would be expected if that

species occurred alone. The data from urine and stool examination surveys in African children living at the Bikita mine (Tables 11D and 12D) show that resistance to S. capense infections is acquired early in life, with a peak of prevalence occurring in the 10 to 12 year age group. However, in the same people there is neither evidence of resistance to the S. mansoni infections nor any evidence of the resistance to S. capense having any influence on incidence of S. mansoni.

It has already been shown that a low incidence of either species will delay or prevent the development of resistance and in this community it is apparent that although the incidence of S. capense is sufficiently high to stimulate the development of resistance to that species, the incidence of S. mansoni would appear to be too low to do so. If, however, the development of resistance to S. capense had conferred any protection against S. mansoni infection, it would be expected that the prevalence and incidence of S. mansoni would have decreased with that of S. capense.

The fact that the prevalence of S. mansoni, as detected by stool examination, did not decrease after the age of peak prevalence of S. capense, indicates a lack of protection to S. mansoni infections despite the development of resistance to S. capense infections; there is a lack of cross resistance between the species from S. capense to S. mansoni. In the community of the Chipoli Estate, the incidence of both S. mansoni and S. capense is sufficiently high for both species to stimulate resistance independently. It is unfortunate that no area was found in Rhodesia where the incidence of S. mansoni was high enough for the development of resistance, but where incidence of S. capense was too low to do so. Therefore, it can only be hesitantly presumed that resistance to S. mansoni confers no pr tection against S. capense infections.

This indication of species specificity of resistance can be supported by the examination of S. mansoni infections in people showing resistance to S. capense. If resistance acquired as a result of infection by one species affords a protection against

infection by a second species, it would be expected that the infections of the second species which do occur, would occur only in those people who had not yet developed resistance against the immunising species.

Fig. 15 presents, in the form of a histogram, the age prevalence relationship of S. capense infections in the communities of the Triangle and Hippo Valley Estates. It may be assumed that, without the influence of resistance, the prevalence would have increased with advancing age; at the very least, it would have remained, in older people, at the level reached at the age of peak prevalence, as represented by the line of expected prevalence E. The difference between the observed level of prevalence and the expected level E, represents the number of people who have developed sufficient resistance to overcome or inactivate the infections. The people still passing eggs must be those who have not developed sufficient resistance to overcome their infections. If resistance has an inter-species influence it would be expected that any infections of S. mansoni which occur would be in those people who are still showing active infections of S. capense. In the age group 21 to 40 years, the prevalence of S. capense infections is 33 percent, and 41 percent have apparently developed sufficient resistance to have overcome their initial and any subsequent infections. This latter group of 41 percent, being resistant to S. capense infections, would also be free, or virtually free, of S. mansoni infections, if the resistance against S. capense also afforded protection against S. mansoni. In this age group, 24 people were found to be passing S. mansoni eggs, and if there is an inter-species protection, the majority of these people should have been in the 33 percent who had not developed resistance and who were still passing eggs, i.e. the majority of S. mansoni infections would have occurred as double infections with S. capense. In fact, only 11 of 24 S. mansoni infections in this group occurred as double infections. Conversely, if resistance is species specific and there is no inter-species influence, the S. mansoni infections should be

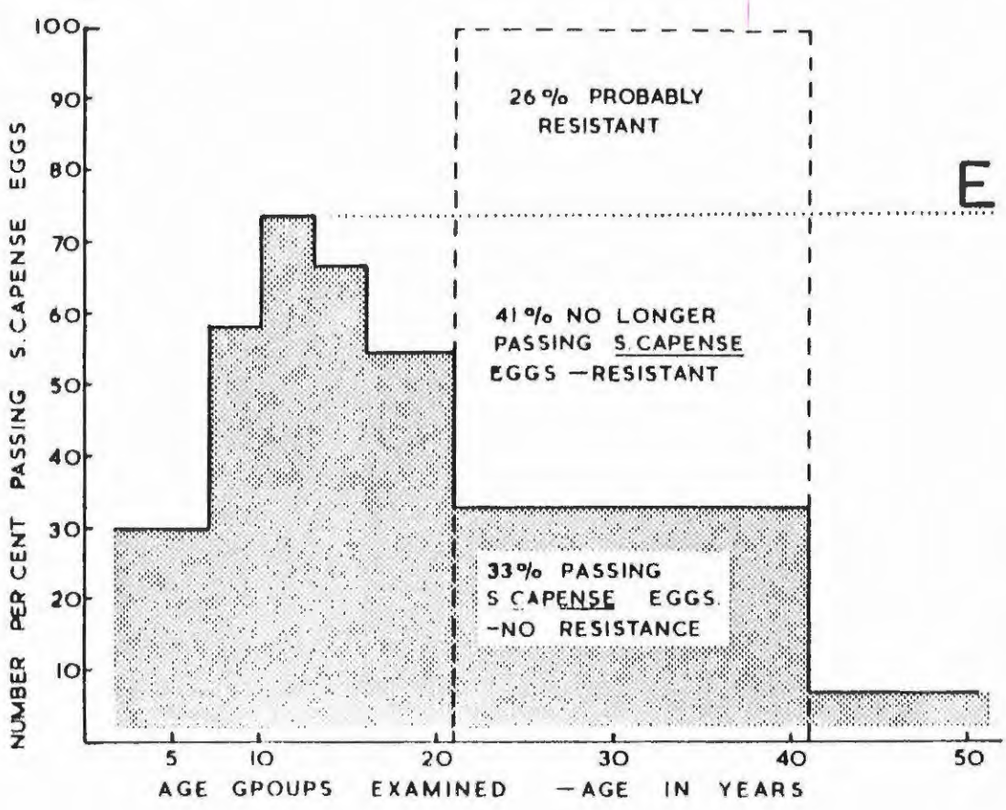


Figure 15. Age prevalence relationship of S. capense infections in the community of the Triangle and Hippo Valley Estates.

randomly distributed through the population, without regard to the distribution of S. capense infections. However, as the proportion of S. capense infections increases, there would be more double infections distributed by chance. Therefore, in the same age group, 33 percent, or 8 of the 24 S. mansoni infections would be expected to occur as double infections with S. capense.

If resistance to S. capense conferred resistance to S. mansoni, the proportion of S. mansoni infections occurring as double infections would increase beyond the proportion of the community showing S. capense infections. However, if resistance is species-specific, the probability of S. mansoni infection of an individual would be independent of whether or not that individual is infected with S. capense.

These concepts may be expressed in ratios as follows:

If the resistance is species-specific the ratio of double infections (d) to single S. mansoni infections (s) would then be the same as the ratio of the number of people showing active S. capense infections (i) to the number of people free of S. capense infection (f).

$$\text{Therefore} \qquad \frac{d}{s} = \frac{i}{f}$$

$$\text{or} \qquad \frac{d}{d+s} = \frac{i}{i+f}$$

$$\text{and} \qquad \frac{d(i+f)}{i(d+s)} = 1$$

but i + f can be taken as the total number of people examined (e) and therefore

$$\frac{de}{i(d+s)} = 1 \text{ (unity)}$$

If there is an inter-species influence of acquired resistance, the proportion of S. mansoni infections occurring as double infections with S. capense would increase with age as resistance increases, and the ratio $\frac{de}{i(d+s)}$ would become greater with advancing age.

Table 18 gives the data obtained from surveys in the communities of the Triangle and Hippo Valley Estates, the Bikita mine, the Turk mine, the Golden Valley mine, and the Arcturus mine. The columns give the values of e, i, d, s and $\frac{de}{i(d + s)}$ for four age groups for each community.

The results show that the values for $\frac{de}{i(d + s)}$ are significantly greater than unity in the majority of areas, but that these values do not increase with age. The fact that they do not increase with age supports the previous evidence that resistance in man acquired as a result of S. capense infections conveys little or no protection against subsequent exposure to S. mansoni. It also indicates that the decrease in prevalence of S. capense is not due to a lack of exposure in older age groups since, if this were so, both S. mansoni and S. capense would be found together in only those people who continued to have contact with water and exposure to infection. Since it is suggested that as they grow older, fewer and fewer people are exposed, it would be expected that the proportion of double infections would rise. It has been demonstrated that this does not happen.

The fact that these values of $\frac{de}{i(d + s)}$ are consistently greater than unity, although they do not increase with age, can be explained by any or all of the following reasons:

(a) The ratio ignores the number of people who fall above the line E (Fig. 15), including those who appear to be naturally resistanct to all schistosome infections. These would weight the values to give a ratio above unity.

(b) There must surely be some individuals or groups of individuals who by inclination, habit or by nature of occupation, have greater contact with

TABLE 18.

Distribution of S. mansoni infections in relation to S. capense infections in six communities in Rhodesia.

		No. examined c	No. passing S. capense eggs i	No. passing both S. mansoni and S. capense eggs d	No. passing S. mansoni eggs only s	$\frac{de}{i(d+s)}$
Triangle & Hippo Valley Estates	: Up to 6 years old	117	35	8	6	1.91
	7 - 12 " "	104	67	19	11	0.98
	13 - 20 " "	148	86	17	10	1.08
	Over 20	353	87	11	21	1.39
	TOTAL:	722	275	55	48	1.40
Bikita Mine	: Up to 6 years old	58	9	0	2	0
	7 - 12 " "	172	82	6	2	1.57
	13 - 20 " "	155	75	2	8	0.41
	Over 20	42	6	1	3	1.75
	TOTAL:	427	172	9	15	0.93
Turk Mine	: Up to 6 years old	42	4	0	0	0
	7 - 12 " "	176	23	3	7	2.30
	13 - 20 " "	26	8	0	0	0
	Over 20	218	17	0	3	0
	TOTAL:	462	52	3	10	2.05
Golden Valley Mine	: Up to 6 years old	141	8	0	10	0
	7 - 12 " "	74	13	2	7	1.26
	13 - 20 " "	12	3	2	0	4.0
	Over 20	93	12	1	12	0.60
	TOTAL:	320	36	5	29	1.31
Arcturus Mine	: Up to 6 years old	94	11	1	4	1.71
	7 - 12 " "	128	59	22	10	1.49
	13 - 20 " "	85	53	21	8	1.16
	Over 20	157	34	10	16	1.78
	TOTAL:	464	157	54	38	1.73
Totals	: Up to 6 years old	452	67	9	22	1.96
	7 - 12 " "	654	244	52	37	1.57
	13 - 20 " "	426	225	42	26	1.17
	Over 20	863	156	23	55	1.63
	TOTAL:	2,395	692	126	140	1.64

infected water throughout their lives, e.g. in African people, girls are given domestic duties, including fetching and carrying of water, washing of dishes and laundry, from a very early age. Throughout their lives they would continue to be exposed more than the boys and men who do not have exposure forced on them as a daily routine.

(c) It is possible that some people are generally more susceptible to infection or penetration by cercariae.

The presence in any age group of any community of a proportion of such people who, because of greater exposure or greater susceptibility, are more likely to become infected with either or both species of Schistosoma would result in the value for $\frac{de}{i(d + s)}$ being considerably greater than unity. (Refer Appendix 1, page 154).

Comparison of Results of Specimen Examinations with Serological or Immunological Tests.

Further evidence to support the existence and development of resistance in man can be derived from a comparison of the results of surveys undertaken by the examination of stool and urine specimens with the results of tests for detectable antibodies in the same people. Two such surveys have been conducted in the course of the present study; these were in the communities of the Golden Valley mine and the R.A.N. mine. A few people in each of the age groups were unable to produce urine or stool specimens. In very young children, administration of skin tests proved difficult. There are, therefore, some discrepancies in the numbers examined or tested by the two methods. In the great majority of cases, the same individuals were tested by both methods. The results are given in Tables 19 and 20, and Figs. 16 and 17.

Table 19 and Fig. 16 show the results of specimen examination

TABLE 19.

The Golden Valley Mine: Comparison of intradermal tests with specimen examination surveys in determining the age-prevalence relationship for *Schistosoma* spp.

	Age Groups Examined						
	0-3	4-7	8-11	12-20	21-40	Over 40	Total
Stool and urine examinations:							
Number of people examined	97	113	91	45	113	75	534
Number of people passing either *S. capense* or *S. mansoni* eggs	10	18	27	20	33	10	118
Number per cent passing eggs	10	16	30	44	29	13	22
Intradermal tests:							
Number of people tested	95	116	96	49	140	106	602
Number showing positive reactions	9	43	58	38	121	90	359
Number per cent showing positive reactions	9	37	60	78	86	85	60

TABLE 20.

The R.A.N. Mine: Comparison of fluorescent antibody tests, intradermal tests and specimen examination surveys in determining the age-prevalence relationship for infections of *Schistosoma* spp.

	Age Groups Examined								
	Under 4	4-6	7-9	10-12	13-15	16-20	21-40	Over 40	Total
Stool and urine examinations:									
Number of people examined	12	31	37	21	1	21	79	72	274
Number of people passing either *S. capense* or *S. mansoni* eggs	0	9	11	9	1	12	36	12	90
Number per cent passing eggs	0	29	30	43	-	57	46	17	33
Fluorescent antibody tests:									
Number of people tested	15	42	46	23	2	22	83	75	308
Number showing positive reactions	4	21	26	19	2	20	65	44	201
Number per cent showing positive reactions	27	50	57	83	-	91	78	59	65
Intradermal tests:									
Number of people tested	13	29	38	21	1	20	79	73	274
Number showing positive reactions	3	7	18	18	1	19	72	65	203
Number per cent showing positive reactions	23	24	47	86	-	95	91	89	74

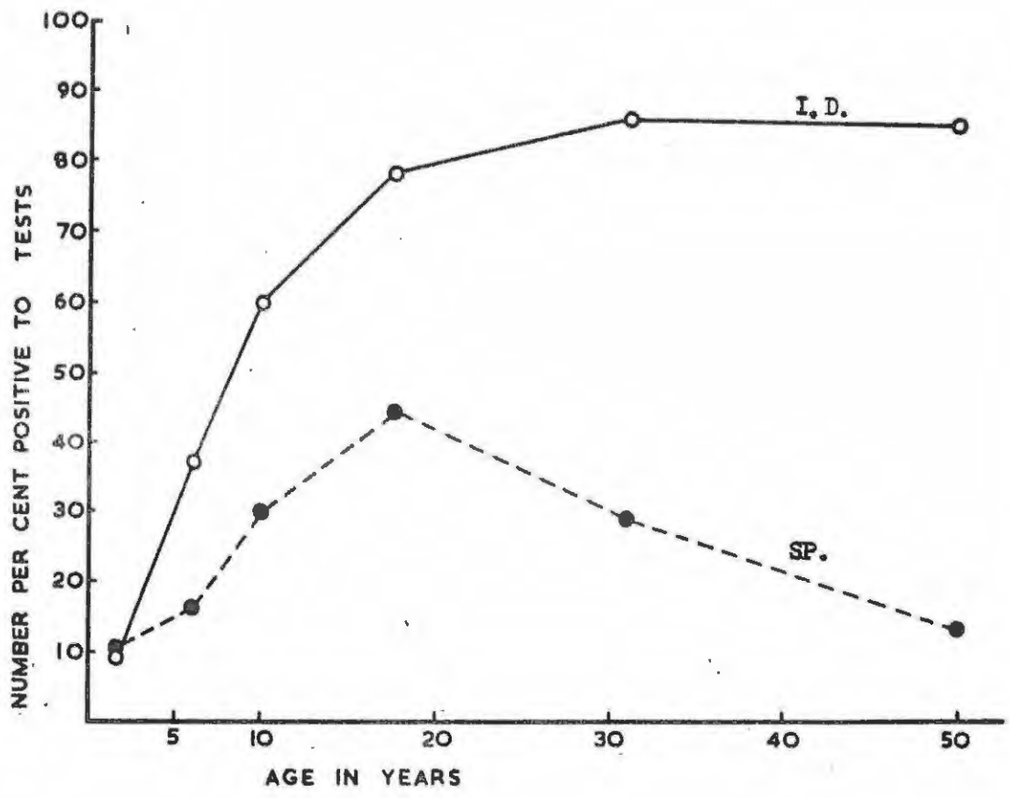


Figure 16. Comparison of age-prevalence of S.capense and S.mansoni infections assessed by specimen examination (SP.) with results on intradermal tests (I.D.) in the community of the Golden Valley Mine.

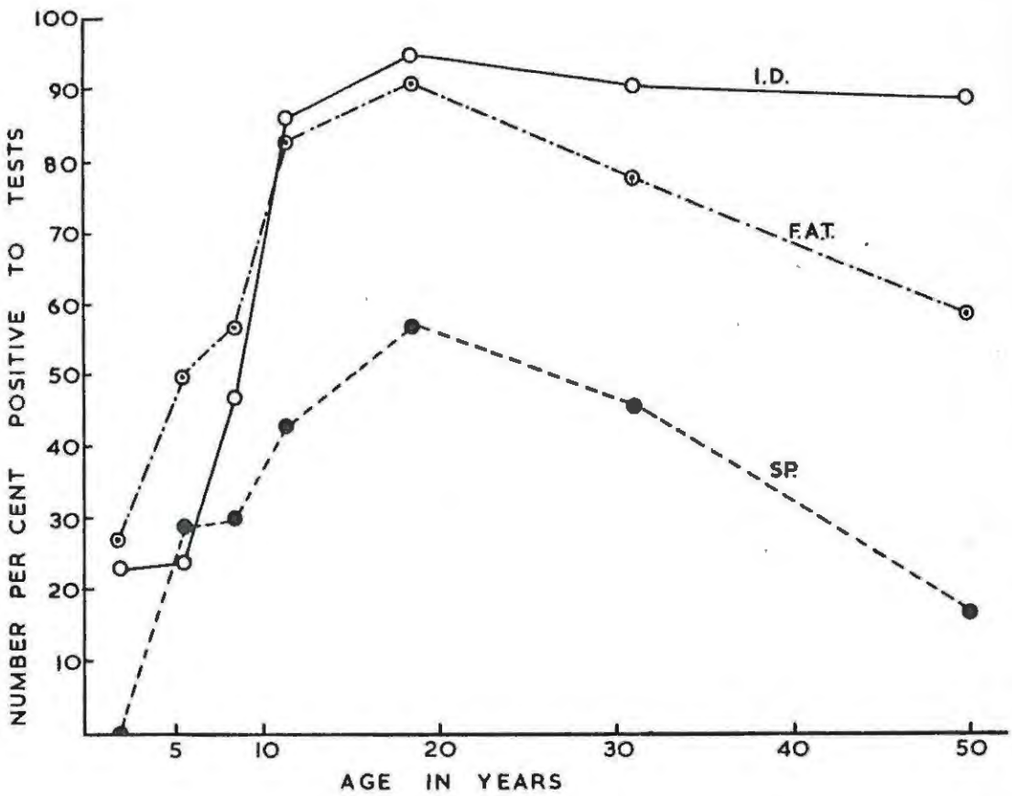


Figure 17. Comparison of age-prevalence of S. capense and S. mansoni infections assessed by specimen examinations (SP.) with results of intradermal tests (I.D.) and fluorescent antibody tests (F.A.T.) in the community of the R.A.N. mine.

surveys and intradermal testing at the Golden Valley mine. This is an area of low prevalence, and the proportion of people passing eggs of either S. capense or S. mansoni reaches a peak of 44 percent in the age group 13 to 20 years. 45 people in this age group were examined, and 20 (44 percent) were found to be passing S. capense or S. mansoni eggs.

In the very young children, under 4 years old, the prevalence based on specimen testing was slightly higher than the prevalence by intradermal testing; this is to be expected since it is known that the skin test lacks sensitivity in the very young (Sadun, personal communication). In the age group 4 to 7 years, the prevalence by skin testing is higher than that shown by specimen examinations, and thereafter there is a progressively greater difference between the two methods of testing as age increases. After the age of peak prevalence, this difference is exaggerated as the number of people passing eggs drops. In the adults over the age of 40 years, only 13 percent of the people tested were passing eggs, but 85 percent of these same people gave positive skin test reactions.

Table 20 and Fig. 17 show the results of specimen examination surveys, and intradermal and fluorescent antibody testing in the same people. A similar pattern of prevalence was seen. The specimen examination surveys showed that a peak of 57 percent was reached in the age group 16 to 20 years, after which the expected falling off of the numbers of people passing eggs was apparent, until in the adults over 40 years old, only 17 percent were still passing eggs of either S. mansoni or S. capense. The intradermal tests gave positive results in 95 percent of the people in the age group 16 to 20 years, and there was subsequently only a very slight fall in prevalence to 89 percent in the adults over the age of 40. The results of the fluorescent antibody testing were similar to those of the intradermal tests up to the age group 16 to 20 years at which age 91 percent were giving positive

reactions. However, after this age, there was a decrease in the proportion of people with positive fluorescent antibody reactions, until, in the adults over 40 years old, only 59 percent showed positive.

Fig. 17 shows that there is again a progressively greater divergence between the results of specimen testing and serological testing. This divergence with increasing age is regular up to the age of peak prevalence, but after this age, the rapid decrease in the numbers of people passing eggs exaggerates the divergence.

Since it is known that detectable antibodies can persist for a considerable, but not necessarily indefinite, period after cure, whether or not this cure is spontaneous or chemotherapeutic, it can be presumed that both the fluorescent antibody test and the intradermal test will detect not only active, but also cured infections, or inactivated infections where no eggs are being passed. Repeated specimen testing aimed at the detection of both S. mansoni or S. capense eggs in the urine or stool will detect only the active infections. It can be presumed therefore, that the differences in the prevalence, as determined by the fluorescent antibody test or the intradermal test on the one hand, and by specimen examination on the other, represents the proportion of inactive or cured infections. In areas such as the two under discussion, where very few of the infections are cured by chemotherapy, the cause of these differences may be ascribed to spontaneous cure or inactivation of the worms.

The divergence between the results of the specimen testing and the immunological testing before the age of peak prevalence suggests that inactivation of the worms occurs very rapidly in some people, but that reinfection can take place. However, with advancing age, more and more children are able to overcome an existing infection and resist subsequent exposure to reinfection. The longer a person has been infected or exposed to reinfection, the less reinfection will occur, until the age is reached where

the numbers of people who overcome their infections equals and then exceeds the number who are reinfected. From this age the prevalence, based on detection of eggs, will decrease with advancing age. However, as long as any antigen either from inactivated but still living worms, or from eggs deposited in the tissues, remains in the body, antibodies will be detectable. There is only a slight falling off of the proportions of people showing apparent infection by either of the immunological tests.

A further comment can be made on the age-prevalence of infections as assessed by immunological tests. In neither of the areas examined did the proportion of people positive to the immunological tests exceed 95 percent. This has been confirmed by two other series in African communities (Clarke et al., unpublished data). It appears therefore that up to 5 percent of the community never show detectable antibodies. The error in the negative results of the fluorescent antibody test has been shown to be low, and it is possible that portion of this 5 percent of the people have never contracted the disease despite probable exposure. This small group might then possess a protection against infection which is not the result of previous infection. Such a protection could not be regarded as an actively acquired resistance, and it would be natural (passive) resistance, according to Boyd's classification (Kagan, 1958). The results of the survey at the Chipoli Estates (Table 12A, Fig. 9A) indicate that where the people are constantly exposed to massive infections, such natural (passive) resistance, and actively acquired resistance, may be partially overcome by the excessive exposure.

4. The Comparison of Prevalence of S. capense Infections before and after a period During which Transmission has been Partially Prevented.

Partial or total interruption of transmission of Schistosoma spp. can be effected by chemical control of snails, and the

comparison of the prevalence of S. capense infections in the children before and after a period of control furnishes additional evidence of the influence of acquired resistance.

A satisfactory snail control programme was maintained in the Chikwanda and Zimutu, and in the Serima areas of Rhodesia for a period which included the years 1960 to 1962. The control was based on the use of the chemical molluscicide "Bayer 73" (the ethanolamine salt of 5, 2'-dichloro-4'-nitro-salicylic-anilide) which was applied to all natural waters at a concentration of 0.5 parts per million if the waterbodies were found to be harbouring vector snails. All water was inspected at six week intervals and if any persisting foci of snails were found, the chemical was applied immediately to eliminate those foci. No quantitative sampling of the snail populations was done, but manual searching for snails demonstrated that successful control of the snails had been achieved, and very few snails could be found during the period under consideration. However, the results of urine examination surveys in the children undertaken in February and March of 1960 before control, and again in the same months of 1962 after two years of control, indicates that a low level of transmission was probably maintained, since some new infections were contracted during the period between the surveys.

The results of the surveys are presented in Tables 21 and 22 and Figs. 18 and 19. The statistical analysis of results is given in Appendix 1 (page 152). In assessing these results no attention has been given to the migrations of people or families into or out of the control area, nor to the children who might have received chemotherapeutic treatment for the disease. It is believed, however, that the numbers concerned constituted only a very small and insignificant proportion of the total numbers examined.

It was anticipated that the most marked effects of the snail control would be apparent in the younger age groups. It was found in fact, that the decrease in prevalence believed to be the result of the control measures, became progressively more marked

TABLE 21.

Results of Urine Surveys for S. capense Infections in African children in the Chikwanda and Zimutu areas in 1960 and 1962.

	Age groups examined							
	Under 4	4-5	6-7	8-9	10-11	12-13	14-15	Total
1960 (Before control)								
Number of children tested	272	412	407	392	145	97	62	1787
Number passing eggs	11	20	127	201	94	62	44	559
Number percent passing eggs	4	5	31	51	65	64	71	31
1962 (After control)								
Number of children tested	61	86	367	504	397	441	234	2090
Number passing eggs	0	3	95	203	180	205	132	818
Number percent passing eggs	0	3	26	40	45	46	56	39

TABLE 22.

Results of urine surveys for S. capense infections in African children in the Serima reserved area in 1960 and 1962.

	Age groups examined								
	Under 2	2-3	4-5	6-7	8-9	10-11	12-13	14-15	Total
1960 (Before control)									
Number of children tested	-	131	178	204	186	64	47	44	854
Number passing eggs	-	9	9	72	120	54	30	25	319
Number percent passing eggs	-	7	5	35	65	84	64	57	37
1962 (After control)									
Number of children tested	2	54	74	177	228	260	290	207	1292
Number passing eggs	0	0	2	25	53	107	132	119	438
Number percent passing eggs	-	0	3	14	23	41	46	57	34

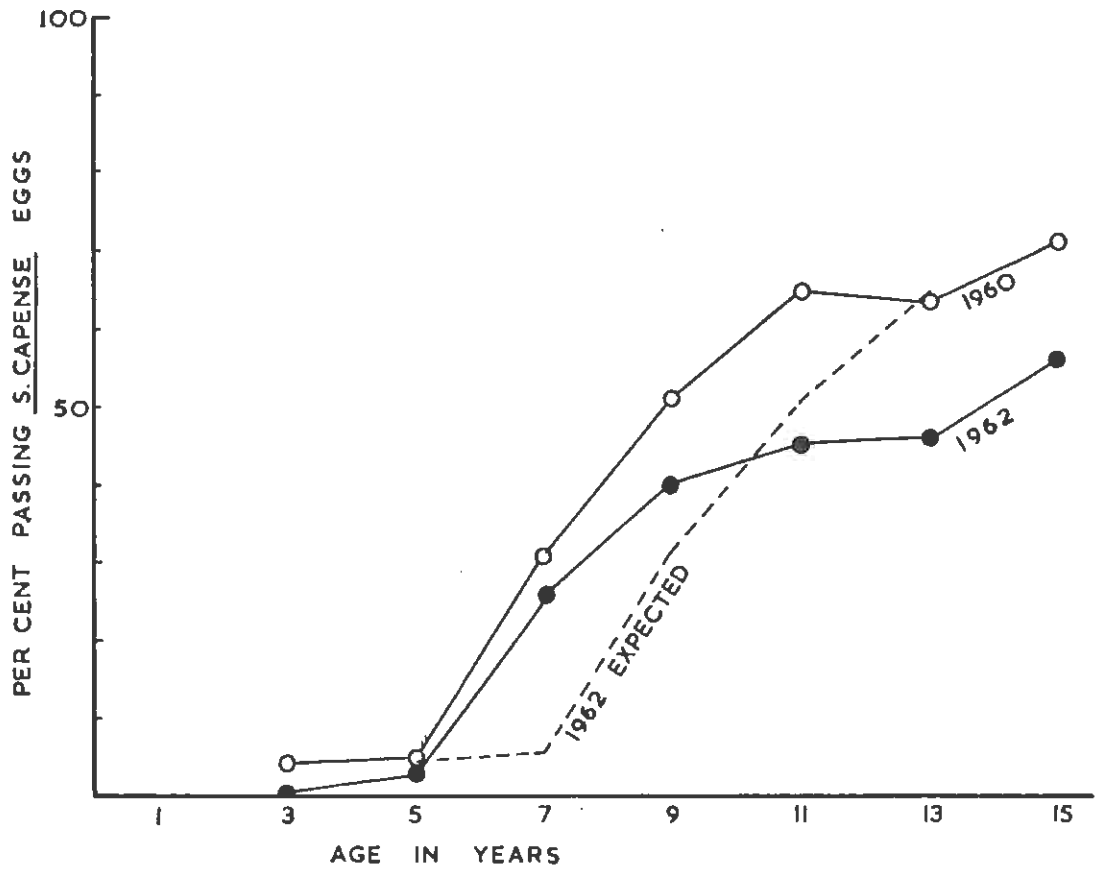


Figure 18. Results of urine examination surveys for S. capense infections in African children in the Chikwanda and Zimutu areas in 1960 and 1962, before and after a period of effective snail control.

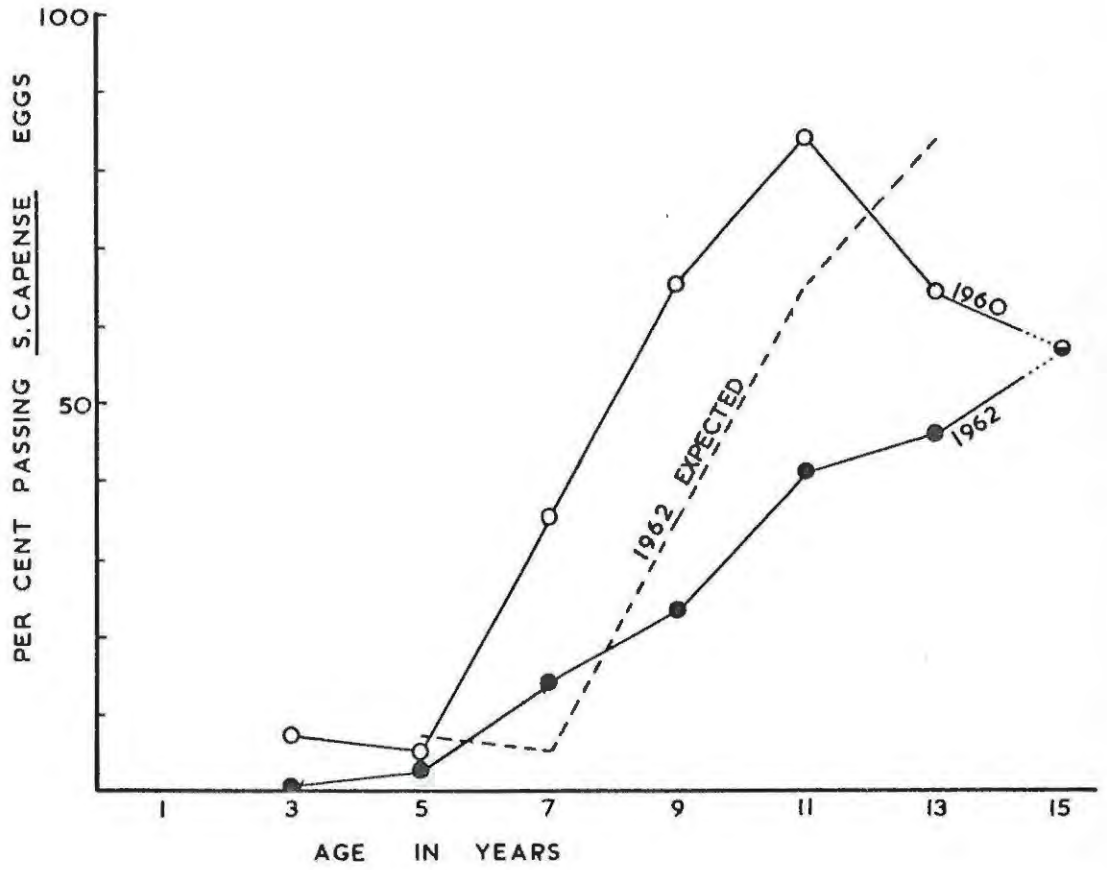


Figure 19. Results of urine examination surveys for S. capense infections in African children in the Serima area in 1960 and 1962, before and after a period of effective snail control.

with increasing age, up to the age of highest prevalence in 1960. Similar patterns of prevalence following control measures were recorded by Berry (personal communication), working in Egypt.

Fig. 18 shows the observed age prevalence of S. capense infections for the Chikwanda and Zinutu areas for both 1960 and 1962. Also illustrated is the "expected" 1962 age prevalence curve which is based on the assumptions that transmission had been totally interrupted during the two year period, and that no resistance, with its consequent inactivation of the infections, had developed. The "expected" 1962 prevalence for each age group under these conditions would then be the same as for that same group of children in the first survey when they were two years younger, i.e. 51 percent of the 8 to 9 year old children showed S. capense infections in 1960; if no new infections occurred, and if none of the existing infections died out, there would still be 51 percent of the children, now aged 10 to 11 years, showing infection.

The decrease in infection was less than expected in the younger age groups and this is probably the result of incomplete control of transmission. The decrease in prevalence in the older children is progressively greater than expected, the difference being greatest at the age of peak prevalence in the first survey. The peak of prevalence in 1962 is in an older age group than in 1960.

The theoretical interpretation of the relationship between the prevalence of infections and the degree of developing resistance, discussed under the section dealing with the age-prevalence section, can be extended to include the differences observed before and after control.

It would appear that where transmission is lowered by snail control, the very young children, with little or no resistance, continue to become infected. The slightly older children develop resistance, largely as a result of infections contracted before the control was initiated; this resistance partially protects

them against the low level of exposure to reinfection, and it overcomes some of the original infections. Still older children have developed stronger resistance, and even more of the original infections are overcome.

Under normal conditions of transmission, the low degree of resistance in young children is overcome by the normal exposure to superinfection, but, as the resistance develops in strength, the extent of superinfection is lessened until a balance between acquired resistance and reinfection or superinfection is reached. Where the extent of exposure is decreased by the control measures, the developing resistance can overcome the existing infections or the subsequent mild reinfections earlier in life, with the result that the fall in prevalence is greater than expected in the older children.

The effects of the control measures will not only be reflected in the decreased prevalence of infections, but also in decrease in the intensity of the infections, because the individuals will be exposed to smaller numbers of cercariae. It must be expected therefore, that with prolonged control, acquired resistance will take longer to develop.

PART IV.

DISCUSSION.

The two main objectives of this study have been to present evidence that resistance to infections of Schistosoma spp. is developed in man, and to draw conclusions on the relationship between acquired resistance and the transmission of the parasites.

The experiments reported in this study, and the reports of other authors have clearly demonstrated that animals develop a resistance to infections of S. mansoni. Some indications have been obtained on factors which influence the rate and degree of resistance developed in animals. Conclusive proof of the development of similar resistance in man would only be obtained from comprehensive experimental infections of human volunteers, but such experiments are impractical. It is therefore necessary to support the limited and inconclusive data from two small series of human volunteer infections with conclusions derived from the indirect evidence that man is capable of immunological responses to infection, since detectable antibodies are produced, and from extensive epidemiological data. Once the conclusion is drawn that resistance is developed in man, then analogies between man and animals are more justifiable.

Resistance in Animals.

The results reported in this study demonstrate that laboratory mice are able to develop resistance to infections of S. mansoni. Other workers have reported similar results, and they indicate that laboratory animals also develop resistance to S. japonicum. Little is known of such protection against infections of S. haematobium or S. capense but there is no reason to suppose that animal host responses to these species is any different. The protective response is probably an immunological resistance to the infection, and it is manifested in the development in the host of a protection

against reinfection or superinfection, and also in the ability of the host to overcome, partially or entirely, the original infection. In the experimental series conducted in the course of this study, the resistance to reinfection or superinfection in laboratory animals was demonstrated by the great reduction in the number of invading parasites of challenge infections which were able to mature. Similar results have been reported by other workers, and they have stated that the resistance may also be demonstrated by stunting of those worms which do mature, by a decrease in the pathogenicity of the subsequent infections, and by the ability of the host to survive exposure to infection which would be lethal to the non-immune host. The ability of the host to overcome the original infection has been demonstrated by the decrease in the numbers of eggs passed by the host and by the apparent death of some or all of the worms, after the infection has been present for some time.

It is apparent from the results obtained that resistance in the experimental mice was developed more rapidly and more strongly from active infections where both male and female worms were present. This is in accord with the reports of other workers, although Vogel and Minning (1953) and Smithers (1962a) reported resistance after infections with one sex only. The results of Series 4 also indicate that some resistance was developed by the mice exposed to single sex infections, but this resistance was not marked. Stage specificity of antigens and detectable antibodies has been reported by Oliver-Gonzalez (1954), and it can be presumed that any protective immunological response would be the result of stimulation by a large number of different antigens. It is therefore probable that maximum resistance would require percutaneous cercarial penetration, the infection maturing with both sexes of worm present, and active egg-laying by the worms.

The experimental immunisation challenge infections have indicated that the rate and degree of resistance developed in mice as a result of one or more exposures to infection with S. mansoni are influenced by the weight of the first exposures, and by the pattern of these

exposures. It is apparent that a very light initial exposure will result in only weak or delayed resistance. A very heavy initial infection or series of infections apparently causes an "immunological paralysis" where the development of resistance is inhibited by the weight of the infection. The resulting resistance, if any, is again weak. The strongest resistance was developed after a single moderately heavy exposure; with this resistance developing rapidly. Most of the reports by other authors (Thompson, 1954; Lurie and de Meillon, 1957; Kagan, 1958) indicated stronger resistance following repeated light exposures to infection. However, these observations were incidental and they were not based on controlled comparative experiments involving immunisation and challenge infections.

The mechanism of resistance even in laboratory animals is not fully understood, although it is apparent that resistance is both cellular and humeral in nature. von Lichtenburg and Ritchie (1961) demonstrated cellular resistance in rhesus monkeys exposed to S. mansoni and there can be little doubt of the accuracy of their findings. There is less proof of a circulating antibody protection against infection, although there is considerable evidence of such a host response. The immobilisation of schistosomulae reported by Newsome and Robinson (1956), the indications of passive transfer of immunity reported by Sadun and Lin (1959) and Bruce and Sadun (1964), and the indications of resistance resulting from antigenic stimulation (Watts, 1949; Sadun and Lin, 1959), all indicate a humeral response.

Whatever the nature of the resistance, this study and the reports of other workers demonstrate clearly that laboratory animals develop resistance against infections of Schistosoma spp. It has also been shown that the nature of the immunising infeotion or infections influences the course of the development of resistance.

Resistance in Man.

Direct proof of the development of resistance in man is not available, and the limited and inconclusive data from two small series of human infection experiments must be supported by the mass of indirect evidence from extensive epidemiological data and from demonstrated immunological activity in man.

The two small series of human infection experiments, although they give some evidence of acquired resistance, are of more importance in the demonstration of natural resistance to species of parasites not usually infecting man. Fisher (1934) reported on the voluntary re-exposure of six men to infections of S. intercalatum, a species which does not usually infect man. However, in the series conducted in Rhodesia, which was completed before the publication of the paper by le Roux (1958) on the nomenclature of the species of Schistosoma, some of the worms were identified by Alves (personal communication) as being S. haematobium. If the identification by Alves was correct, he was referring to the southern African species, accepted in this work as being a separate species S. capense.

The immunological activity in man is indicated by the demonstration of detectable antibodies by serological tests, and by the demonstration of antibodies, probably protective antibodies, by the immobilisation of schistosomulae. A positive relationship between the presence of detectable antibodies and protective antibodies has not been established but it is logical to assume that the presence of detectable antibodies makes it probable that protective antibodies will also be present, either concurrently or subsequently.

The epidemiological evidence of the development of resistance is based on patterns of decreasing prevalence of infections, and on decreasing egg production, in the older age groups. It is also indicated by comparison of age prevalence relationships as assessed by specimen examinations or by immunological tests, and by comparison of results of specimen examination surveys before and after periods during which transmission was partially interrupted.

The popular theory that the decrease in age prevalence of infection and in egg production in older people is the result of mechanical fibrotic obstruction of egg passage through the walls of either the bladder or the bowel, is totally unacceptable. It is also unacceptable that these decreases with advancing age are the results of natural senescence of the worms, which have been shown to have a life span of over 25 years in several recorded instances. The results reported in this study, and by other workers. show that the only acceptable conclusion is that the death or the inactivation of the worms of the infection are the result of host response, which affords a protection or resistance against future infection.

Recent incomplete investigations by Gelfand et al. (1964, in press) indicate that there is a transfer of detectable antibodies from mother to child during pregnancy, since antibodies have been detected by the bilharzial fluorescent antibody test in the sera of new-born infants of infected or previously infected mothers. Further investigations with more specific and reliable tests, possibly the complement fixation test, are required to confirm these reported results. If protective antibodies are also present in infants as a result of congenital passive transfer, it would explain the scarcity of infections in young infants who, under conditions currently prevailing in Rhodesia, are often exposed to infection from a very early age when they are bathed in natural water.

Reference has been made in this work to records of individuals in whom resistance apparently may persist after chemotherapeutic treatment. These records and the data from the epidemiological surveys indicate that the development of resistance in man may be in two phases. In the early stages of infection, the resistance is probably in the form of a premunition, where the protection conferred on the host is dependent on the presence of a living infection. At this stage, the death of the worms after chemo-therapy would result in the loss of resistance, and the host would

soon be susceptible to reinfection. However, it appears that in time, the premunition is replaced by a true immunity, where the resistance of the host is a true host response which is no longer dependent on the continued presence of living worms or of antigenic material. This immunity would depend on the very rapid production of antibodies when the host is exposed to reinfection. The development of such immunity is shown by the persistence of protection after chemotherapy reported by Vogel and Minning (1953) in rhesus monkeys, and by observations of non-recurrence of infection, despite almost certain re-exposure, in people treated for long-standing infections.

From the available data of the human infection experiments, and from the mass of evidence from epidemiological surveys and from demonstrated immunological activity in man, it is possible to draw the firm conclusion that man is capable of developing resistance to infections of Schistosoma spp.

Once this conclusion is reached, further conclusions on the development of resistance in man can be made.

Conclusions from Epidemiological Data.

The epidemiological surveys have afforded evidence to support three important assumptions relating to the development of resistance in man. These assumptions are that there are racial differences in susceptibility and the ability to acquire resistance, that acquired resistance may be species specific, and that the rate and degree of resistance developed are influenced by the patterns of transmission to which the people are exposed.

There is some evidence that resistance, both natural and acquired, is more active in Africans than it is in Europeans. The majority of clinical observers agree that bilharziasis is more severe in Europeans, in whom even apparently light infections produce clinical disease. This difference in susceptibility and resistance must be considered in determining the policies and priorities of control programmes.

As in laboratory animals, it is clear that in man there are pronounced individual variations in susceptibility to infection, and in the host response and the subsequent degree of resistance which follows the infection. It is probable that the acquired resistance in an individual is in any case influenced by a number of other factors; it may be weakened by other concurrent illness, or, as reported by Vogel (1958) in rhesus monkeys, by parturition, or it may be dependent on nutritional status.

The evidence of species specificity of resistance in man is at present limited. It has been shown that resistance to S. capense does not protect against S. mansoni infection. There is no evidence on the influence of S. mansoni resistance on S. capense infection, and similarly there is no knowledge on possible interspecies resistance with other species of Schistosoma. The lack of protection against S. mansoni in people who are resistant to S. capense infections could be of importance in many countries of Africa, where the development of irrigation is leading to an increase in the prevalence of S. mansoni in people previously exposed to heavy infections only of S. capense.

It has been established that the rate of the development of resistance, and the degree of resistance developed in man are dependent on the nature and extent of exposure to infection. There is, therefore, a relationship between the rate and degree of the development of acquired resistance and the extent and conditions of transmission to which the people are exposed.

The Relationship between Acquired Resistance and Transmission.

The epidemiological data shows that the extent of resistance developed in the people of a community is influenced by the patterns of transmission to which they are exposed. In the communities examined, the strongest resistance to S. capense infections was apparently developed in the people of the Arcturus and Iron Duke mines, where, after the ages at which the peaks of prevalence and of average egg production occurred, both the prevalence and the egg production decreased rapidly. Both these mining communities

are in areas where transmission would be expected to be intense during two relatively short seasons, but low during the remainder of the year. This conforms with the opinions of Gerber (1952) that maximum resistance develops when a moderate initial exposure is followed after an appreciable period of time, by regular re-exposures to infection. He suggested that the intervals between the initial and subsequent exposures to infection should extend to cover whole seasons of very low transmission. Weak resistance was shown by the people of the Turk mine, where the fall in prevalence and egg production was less rapid. In this community, where the expected pattern of infection is one of seasonal low-level exposures, it is presumed that the original infections are too light to develop full resistance. However, in the community of the Chipoli estate, where the people are exposed to intense perennial transmission, it is apparent that resistance is again weak. In this case it is probable that the development of immunity is prevented by the very heavy exposures to infection in early childhood.

In bacterial or virus disease, it is known that it is in the early years of life that immunogenic processes are most active, and it is probable that the same is true for parasitic infections. If these processes are prevented by the heavy infections, the resulting "immunological paralysis" would persist for life, and resistance, if any, would be weak. As a result, a large proportion of the people, even of the older adults, were found to be passing eggs. It is apparent that the most effective resistance is developed in communities exposed at intervals or seasons to moderately heavy infections. Too low or too high a level of transmission will limit the development of resistance in the people.

Patterns of Transmission.

The patterns of transmission are governed by the interaction of a number of different factors. The most important of these are the stability and permanence of the waterbody, the water temperature, and the density of the human population having contact with the waterbody.

Dense snail populations are usually found only in stable water-

bodies. Even if all other conditions are suitable, snails will seldom be found in large numbers in waterbodies liable to excessive flooding during the rains, or to drying out during the winter and early summer. Shiff (1964a, 1964b) observed that this is particularly true for the snail host of S. mansoni, Biomphalaria pfeifferi, which can only survive in permanent water. Bulinus (Physopsis) globosus, the main host snail of S. capense in Rhodesia, is able to withstand desiccation, but nevertheless, dense populations of this snail are seldom found in temporary waters.

Temperature has a pronounced effect on the infectivity of any waterbody, and on the level of transmission. High water temperatures lead to rapid reproduction by snails, and the proportion of young, susceptible snails will be high (Shiff, 1964a, 1964b). Water temperature also influences the rate of sporocyst development, the abundance of algal food for the snails, and the extent of human contact with the water. All these result in a high infectivity of the waterbody when temperatures are high. Pitchford and Visser (1962) demonstrated seasonal differences in the transmission of S. mansoni by recovering schistosome worms from groups of mice which had been immersed in naturally infected water in a highly endemic area. Although they did not correlate the seasons of transmission with the temperature of the water, they did find the highest transmission occurring during summer. During the winter, few if any of the mice became infected.

The density of the human population dependent on the water for domestic or recreational purposes will also affect the level of transmission. This was shown for S. mansoni infections by Pitchford (1958) when he demonstrated that prevalence of these infections increased with the human population concentration.

Four distinct patterns of transmission can be found in Rhodesia.

In areas more than 1,000 m. above sea level, transmission is limited or absent during the winter months when the water temperatures are low. Transmission is therefore seasonal and limited to the summer. In some parts of the high plateau, where these seasonal

conditions of transmission occur, the transmission in summer is at a high level. In other parts, particularly in the south-west of the country, the unstable waterbodies and the low density of human settlement prevent high summer transmission.

In the hot low-lying areas of Rhodesia, the water temperatures, even during the winter, are sufficiently high for continued transmission. Where waterbodies are not stable, and where human settlement is sparse, this perennial transmission is maintained only at a low level. Where the waterbodies are stable and the human settlement is dense, as on the irrigation estates, perennial, intense transmission will occur.

The pattern of high summer transmission alternating with winter seasons of little or no transmission results in strong resistance in the community. Examples of this are found in the communities of the Iron Duke, the R.A.N. and the Arcturus mines.

Where a community, such as the Turk mine, is exposed to a low level of transmission which is even then limited to the summer months, the resistance acquired by the community is weak.

Perennial transmission at a high level is found in the irrigation systems of the hot low-lying areas of the country. An example is the Chipoli estate. In these areas, the prevalence of infections of both S. mansoni and S. capense is high, and the resistance acquired by the communities is weak. The Triangle and Hippo Valley estates also have conditions suitable for intense perennial transmission. However, because they are newly developed estates, and because many of the people tested are new to the area, the results of the epidemiological surveys do not reflect the expected conditions of transmission.

Probable Future Developments.

It is possible to predict the future development of bilharziasis in Rhodesia if no control measures are initiated.

On the high central plateau, where most of the European community is concentrated, the continuing programme of water conservation can

only result in increasing intensity of transmission in the summer months. In the north-eastern regions of this plateau, where summer transmission is already high, it will become more intense. In the south-western regions, where transmission is at present low, even during the summer transmission season, the water conservation measures are creating stable waterbodies. With the increased human population densities resulting from the intensification of agriculture, the summer transmission must increase. In the African population, the prevalence of infection in the children will be high, but the effective resistance developed will result in a decreased prevalence in older people. The European population will be exposed to infections of increasing intensity without the protection of effective resistance. It must be expected that the morbidity due to the disease will increase in all sections of the community, but particularly in the Europeans. Infections of both species will increase in prevalence.

In the hot low-lying areas of the country, human population density is generally low. Except in irrigation settlements, it is likely that incidence and prevalence of the disease will remain low. However, in irrigation settlements, the only logical expectation must be that incidence, prevalence and intensity of infections of both S. mansoni and S. capense will increase dramatically. This will result from the combination of three factors, the stability and permanence of the waterbodies, the high temperatures which are maintained throughout the year, and the dense human settlement. As stated previously, these factors have been shown by Pitchford (1958), Pitchford and Visser (1962) and Shiff (1964a, 1964b) to be important in maintaining a high level of transmission. Resistance acquired under these conditions of transmission will be weak. It must be expected that morbidity or even mortality will increase both in Europeans and in Africans.

The present policy of recruiting labour for the irrigation projects from the low prevalence areas of the western half of the country, where community resistance is low, must result in intense

infections. These infections will occur in the economically productive adults as well as in the children. The resulting morbidity in adults could have an adverse effect on the economy of the irrigation programmes.

Eventual control of the disease will require a combination of all available methods to break the cycle of the disease or to limit the extent of transmission.

This study has emphasised the need for detailed research prior to control. The importance of the influence of acquired resistance on the epidemiology of the disease has been overlooked in the past. On available knowledge it would appear that control should be limited to those areas where transmission of the disease is too high to allow the development of resistance. This would apply only where control is designed to protect the African community; where Europeans require protection, all transmission must be stopped. Epidemiological investigations therefore determine the priorities of control, and the policies of control can only be determined on a sound knowledge of the seasonal patterns of transmission of the disease.

If control is aimed at limiting or eliminating the clinical disease in a community, and not at protecting the individual, there are a number of investigations which are required.

It is necessary to determine the mechanism of the development of resistance. If, in fact, there is developed in man a true immunity which is preceded by a stage of premunition, attempted control by mass chemotherapy could do more harm than good. This would happen if the protection due to premunition was destroyed by the drug treatment, and the individuals were subsequently exposed to heavier infection. The mass treatment of all Africans, a common practice on many Rhodesian farms, cannot be justified.

This study has demonstrated that the patterns of transmission to which the people are exposed influence the levels of acquired resistance developed in the people. More detailed investigations on these influences are required. Such information will be available from

surveys to assess the prevalence patterns in communities exposed to all the different patterns of transmission. Regular surveys in the labourers and their families forming the new communities of the irrigation estates will provide information on the development of resistance in unprotected communities.

The answers to the control and eventual elimination of bilharziasis cannot be expected from laboratory research in non-endemic countries. Laboratory and field investigations in endemic areas must be intensified if the disease is to be controlled, but there is an urgency for these investigations which is not fully appreciated. It is estimated that the irrigated areas of Rhodesia will eventually support over one million people, and the annual income from sugar production alone is expected to exceed £100 million within the next decade. This gigantic enterprise could be jeopardised by bilharziasis.

PART V.

SUMMARY.

(i) This study attempts to assess the importance of immunological resistance against infections of Schistosoma spp. in man and in laboratory animals.

(ii) The development of acquired resistance in white mice is demonstrated by high mortality of worms of challenge infections in immunised mice. The number of worms recovered from immunised and normal mice were compared.

(iii) The mice were immunised by exposure to infection by S.mansoni. The numbers of cercariae used for the immunising infections, the immunising period between the initial infection and the challenge exposure, and the patterns of exposure were varied.

(iv) The results of animal infection experiments showed that resistance was acquired very rapidly in mice exposed to single immunising infections. Multiple immunising infections did not result in effective resistance. Heavy initial infections resulted in high mortality in the test mice, and the survivors showed only low resistance.

(v) There was no apparent stunting of the worms as a result of the acquired resistance. The methods used did not detect any decrease in the number of eggs laid by the worms of the challenge infections.

(vi) The results of experimental infections in rhesus monkeys reported by Jachowski et al.(1963) are discussed. These results showed a decrease in egg production from the animals as they developed resistance. The rate at which the resistance developed depended on the nature of the immunising infection.

(vii) Two series of human volunteer experiments are discussed. One of these was reported by Fisher (1934), and the other was undertaken locally. These experiments indicate that resistance is developed in man.

(viii) The development of detectable antibodies in an infected person is used as evidence of immunogenic activity in the person. If detectable antibodies are produced, it is logical to assume that there is concurrent or subsequent development of protective antibodies.

(ix) Newsome and Robinson (1956) reported the formation of precipitates round schistosomulae incubated in immune serum. This is taken to be evidence of protective antibodies in serum.

(x) Extensive epidemiological surveys were undertaken in several large communities. These showed the development of acquired resistance in man. It was demonstrated by the patterns of prevalence of infection in relation to age, and by the numbers of eggs passed by the people of different age groups. Comparison of specimen examination with immunological tests, and differences in prevalence of infection before and after periods of transmission control, also indicated the development of resistance.

(xi) It has been shown that the rate and degree of the development of acquired resistance in a community are influenced by the patterns of transmission to which the people are exposed.

(xii) Epidemiological evidence has been presented to show that resistance acquired as the result of infection by one species of Schistosoma does not necessarily protect against exposure to infection by another species.

(xiii) The probable future importance of bilharziasis in the large irrigation projects in the hot areas of Rhodesia is discussed. It is believed that the disease in these areas will increase in prevalence, intensity and economic importance. One of the reasons for this will be the low level of acquired resistance in people exposed to heavy infections.

(xiv) It is felt that there is an urgency for intensified research, particularly in these irrigation projects, to ensure that adequate control can be effected before the economy of the large irrigation programme in Rhodesia is adversely affected by the disease.

APPENDIX 1.

STATISTICAL PROCEDURES USED.

Results of immunisation-challenge infections of mice: comparison of numbers of worms recovered from mice exposed to different conditions of infection.

In the interpretation of the significance of the results of perfusion for the numbers of worms maturing from any infections in mice, the transformation $\sqrt{n_i + 1}$ has been used, where n_i is the number of worms recovered from a mouse. This transformation (Snedecor, 1937) was adopted because the numbers of worms recovered appeared to conform to a Poisson distribution as there was a uniform probability for the number of worms in each mouse. Since means were compared, it was not in fact necessary to use any transformation, but since corrections, based on standard deviation values, were made, it was considered advisable to use such transformations.

The results for groups of mice infected under different conditions of exposure have been compared by calculation of the standard error of the difference between the means of the square root transformations of the numbers of worms recovered. A difference between means was accepted as significant when the difference was twice the value of the standard error of difference.

When comparing the results of perfusion of mice of the immunised-challenged groups with the challenge control groups, a correction was needed for the numbers of worms surviving from the immunising infection. The correction for the mean value of $\sqrt{n + 1}$ for immunised challenged groups was derived from the mean and standard deviation values of $\sqrt{n + 1}$ for the equivalent immunised control group. The correction used was:

mean of $\sqrt{n + 1}$ minus 2 x standard deviation of $\sqrt{n + 1}$.

This correction was subtracted from the mean of the transformations of the experimental group of mice, to give a corrected mean.

APPENDIX 1. (Cont.)

Statistical analysis of differences in average S.capense egg production in infected people of different age groups. Results recorded from the Bikita Mine.

The significance of the differences in average egg production for the different age groups examined was analysed by the Kruskal-Wallis one-way analysis of variance method for large samples (Siegel, 1956). This non-parametric test was preferred to parametric tests because it does not require the corrections for heterogeneity of variance which would be necessary for the F test, and because, although the non-parametric test loses some sensitivity, the results give a low order of probability for the null hypothesis and therefore a high degree of sensitivity is not necessary. Table 23 gives the actual S.capense egg counts recorded at the Bikita mine, arranged in age groups. The age groups under 4 years and over 40 years have been omitted because only 1 and 2 people in these respective groups were passing eggs. The table also gives the ranked order for the counts, with ties being given the mean of the ranks for which they have tied. Table 24 lists the ties and the values for T for each tie, where $T = t^3 - t$, t being the number of counts in each tie. The factor T is used in determining the correction required for the ties.

The test is required to demonstrate differences in the means of egg counts in the different age groups.

The formula used is

$$H = \frac{12}{N(N+1)} \sum_{j=1}^{k} \frac{R_j^2}{n_j} - 3(N+1)$$

where H is the parameter measured by the Kruskal-Wallis test, and is the measure of the disparity between the sums of the ranks for each

age group; it is also the likelihood that the samples were drawn from the same population.

k is the number of samples (age groups),

n_j is the number of counts (ranks) in the jth. sample,

N is the number of counts in all the samples ($\sum n_j$),

R_j is the sum of the rank values in the jth. sample,

$\sum_{j=1}^{k}$ stipulates the sum of the k samples.

substituting,

$$H = \frac{12}{169(170)}\left(\frac{504100}{8} + \frac{5890329}{22} + \frac{35176761}{60} + \frac{5643000}{41} + \frac{6901129}{34} + \frac{86730}{4}\right) - 3(169+1).$$

$H = \underline{24.35}$

It is now necessary to correct for ties, using the formula

$$H_c = \frac{H}{1 - \frac{\sum T}{N^3 - N}}$$

Table 24 gives the ties that occurred, and the values $T = t^3 - t$.

Substituting

$$H_c = \frac{24.35}{1 - \frac{4746}{4826640}} = \underline{24.591}$$

H is distributed approximately as chi-square with k - 1 degreos of freedom for large samples.

Where H 24.591, with five degrees of freedom, p is less than 0.001, and the significance of the differences in egg production in different age groups can be accepted.

TABLE 23

Bikita Mine: Counts of S. capense eggs released in urine from 169 people of six age groups.

Age group 4-6		Age group 7-9		Age group 10-12		Age group 13-15		Age group 16-20		Age group 21-40	
Egg counts	Ranks	Egg counts	Ranks	Egg counts	Ranks	Egg counts	Ranks	Egg counts	Ranks	Egg counts	Ranks
0	6.5	30	33.5	0	6.5	0	6.5	0	6.5	10	18.0
30	33.5	30	33.5	0	6.5	0	6.5	0	6.5	170	72.0
120	58.5	30	33.5	0	6.5	0	6.5	0	6.5	330	89.5
380	95.5	60	44.5	0	6.5	10	18.0	10	18.0	720	115.0
580	109.0	150	65.5	0	6.5	10	18.0	20	26.0		
620	111.5	160	68.0	10	18.0	10	18.0	30	33.5		
1450	144.5	290	85.0	10	18.0	10	18.0	30	33.5		
2440	151.0	340	91.5	30	33.5	10	18.0	50	40.5		
		380	95.5	30	33.5	10	18.0	70	48.0		
		510	106.0	60	44.5	10	18.0	70	48.0		
		830	124.0	60	44.5	20	26.0	100	54.0		
		1000	128.0	80	51.0	20	26.0	100	54.0		
		1010	129.0	130	60.5	20	26.0	110	56.5		
		1110	133.5	140	63.0	20	26.0	120	58.5		
		1410	142.5	170	72.0	30	33.5	140	63.0		
		2120	148.0	170	72.0	30	33.5	150	65.5		
		2550	153.5	170	72.0	50	40.5	180	75.0		
		2960	156.0	170	72.0	50	40.5	190	76.0		
		3100	157.0	210	77.0	50	40.5	270	82.0		
		4930	164.0	220	78.0	60	44.5	280	83.0		
		9200	167.0	230	79.0	70	48.0	350	93.0		
		13580	168.0	290	85.0			380	95.5		
				290	85.0	80	51.0	440	100.5		
				310	87.5	80	51.0	450	102.0		
				310	87.5	100	54.0	500	105.0		
				330	89.5	110	56.5	710	114.0		
				380	95.5	130	60.5	750	118.0		
				410	98.0	140	63.0	790	121.0		
				430	99.0	160	68.0	820	122.5		
				440	100.5	160	68.0	1100	131.0		
				530	107.5	240	80.0	1120	136.0		
				530	107.5	260	81.0	1270	141.0		
				600	110.0	340	91.5	2890	155.0		
				620	111.5	460	103.0	3460	158.0		
				700	113.0	470	104.0				
				730	116.5	780	120.0				
				730	116.5	820	122.5				
				760	119.0	880	125.0				
				890	126.0	1020	130.0				
				960	127.0	1110	133.5				
				1110	133.5	1240	138.0				
				1110	133.5	1450	144.5				
				1160	137.0						
				1260	139.5						
				1260	139.5						
				1410	142.5						
				1620	146.0						
				1900	147.0						
				2390	149.0						
				2420	150.0						
				2490	152.0						
				2550	153.5						
				3590	159.0						
				3790	160.0						
				4260	161.0						
				4300	162.0						
				4860	163.0						
				5950	165.0						
				8600	166.0						
				29600	169.0						
Sum of Ranks	R = 710.0	2427.0		5931.0		2375.5		2627.0		294.5	
No. of counts, nj =	8	22		60		41		34		4	

TABLE 24

Table of ties for ranks for egg counts in S. capense infections at the Bikita Mine. Derivation of ΣT for correction factor for Kruskal-Wallis test for variance.

Number of eggs	Number of ties (t)	$T = t^3 - t =$
0	12	1716
10	11	1320
20	5	120
30	10	990
50	4	60
60	4	60
70	3	24
80	3	24
100	3	24
110	2	6
120	2	6
130	2	6
140	3	24
150	2	6
160	3	24
170	5	120
290	3	24
310	2	6
330	2	6
340	2	6
380	4	60
440	2	6
530	2	6
620	2	6
730	2	6
820	2	6
1110	4	60
1260	2	6
1410	2	6
1450	2	6
2550	2	6
		$\Sigma T = 4746$

APPENDIX 1.(Cont.)

Analysis of numbers of infected people, in four age groups, passing more than 201 eggs of S.capense in one hour. Results recorded from Premier Estate.

Table 25 sets out the actual numbers of the people of the Premier estate who were passing under or over 201 eggs of S.capense in one hour urine specimens. The numbers are set out in a contingency table for the chi-square determination.

The null hypothesis tested was that the proportions of people passing more than 201 eggs was the same in all age groups. The expected values for the numbers of people passing more or less than 201 eggs were derived from the total numbers of people examined and the total numbers in each category.

With 3 degrees of freedom, the chi-square value 19.90 gives a probability of less than 0.001.

It can be accepted that the proportions of people passing over 201 eggs differ in the age groups examined.

TABLE 25

Contingency table for numbers of infected people, in four age groups, passing under or over 201 eggs of S. capense. Premier Estate.

	4 - 9	10-12	13-20	21+
Total number passing eggs	47	32	19	33
Observed number passing over 201 eggs	31	16	6	6
Expected number passing over 201 eggs	21.1	14.4	8.6	14.9
Difference	9.9	1.6	2.6	8.9
$\frac{(\text{Difference})^2}{\text{Expected}}$	4.65	0.18	0.79	5.32
Observed number passing less than 201 eggs	16	16	13	27
Expected number passing less than 201 eggs	15.9	17.6	10.4	18.1
Difference	9.9	1.6	2.6	8.9
$\frac{(\text{Difference})^2}{\text{Expected}}$	3.78	0.15	.65	4.38

$X^2 = 19.90$

$n = 3$

P is less than 0.001

APPENDIX 1 (Cont.)

Analysis of results of urine examination surveys in the Chikwanda and Zimutu areas before and after a period of snail control.

Children in eight age groups up to the age of 15 years were examined for S.capense infections in 1960, before any attempt at snail control was made. An intensive programme of snail control, using chemical molluscicides was then instituted, and this programme was aimed at eliminating transmission of the infections. After two years of control, a further survey in the same area was conducted. The results were presented in table 21, and table 26 sets out the chi-square analysis of the results in the form of a contingency table. The two youngest age groups were combined because in each case the number expected to be passing eggs in 1962 was below 5. The expected number was calculated from the proportions of people found to be passing eggs in 1960.

The chi-square value of 68.04 with five degrees of freedom gives p less than 0.001. This shows that the observed differences in prevalence for 1960 and 1962 were significantly different, with the prevalence in 1962 lower than that of 1960.

TABLE 26.

Chi-square analysis of difference of results of urine examination surveys in Chikwanda and Zimutu areas in 1960 and 1962.

	Age Groups Examined						Totals
	Under 6	6 - 7	8 - 9	10-11	12-13	14-15	
1960:							
Number examined	684	407	392	145	97	62	1787
Number passing eggs	31	127	201	94	62	44	559
Percent passing eggs	4.5	31	51	65	64	71	31
1962:							
Number examined	147	367	504	397	441	234	2090
Observed number passing eggs (o)	3	95	203	180	205	132	818
Expected number passing eggs based on 1960 proportions (e)	6.6	114	257	258	282	166	
o - e	-3.6	-19	-54	-78	-77	-34	
$(o - e)^2$	12.96	361	2916	6084	5929	1156	
$\frac{(o - e)^2}{e}$	1.96	3.17	11.35	23.58	21.02	6.96	

Chi-square = 68.04
Degrees of freedom = 5
p is less than 0.001

APPENDIX 1. (Cont.)

Statistical analysis of data on distribution of S.mansoni infections in relation to S.capense infections.

Table 18 (page 114) gives the values for the ratio $\frac{de}{i(d + s)}$ for four age groups in each of five communities in Rhodesia. In this ratio,

e is the total number of people examined,

d is the number of people passing eggs of both species,

i is the total number of people passing S.capense eggs, and

s is the number of people passing S.mansoni eggs only.

If m is the total number of people passing S.mansoni eggs, then $m = d + s$, and the ratio is $\frac{de}{im}$.

It is necessary to test the hypothesis that the number of double infections is greater than would be expected by chance alone.

If S.capense infections and S.mansoni infections are distributed independently of each other, then $\frac{de}{im} = 1$.

On the null hypothesis that the distribution of S.mansoni in relation to S.capense infections is random, where $\frac{de}{id} = 1$, it is possible to calculate the expected values for d and s:

$$\text{expected } d = \frac{im}{e}$$

$$\text{expected } s = \frac{de}{i} - d.$$

These two values for each age group can be compared with the observed values by the chi-square test. Since there is only one degree of freedom, it is necessary to apply Yates' correction.

Thus for the combined figures for all communities, in the

age group up to 6 years,

$$\text{expected d} = \frac{im}{e} = \frac{67 \times 31}{452} = \underline{4.6}$$

$$\text{expected s} = \frac{de}{i} - d = \frac{9 \times 452}{67} - 9 = \underline{51.7}$$

These two values do not give the same total as the observed values, and they must therefore be reduced to do so. The sum of these two values is 56.3, but the sum of the observed values is 31, therefore the corrected values for use in the chi-square test are:

$$\text{expected d} = 4.6 \times \frac{31}{56.3} = \underline{2.53}$$

$$\text{expected s} = 51.7 \times \frac{31}{56.3} = \underline{28.47}$$

These two values may be compared with the observed values of 9 and 22 respectively, and a chi-square value of 15.34 is obtained. With one degree of freedom, this gives p less than 0.001.

Applying similar procedures to all age groups:

Age group up to 6 years, chi-square 15.34, p is less than 0.001.
7 - 12 years, chi-square 40.78, p is less than 0.001.
13 - 20 years, chi-square 3.85, p is 0.05.
over 20 years, chi-square 21.22, p is less than 0.001.

This demonstrates that double infections occur more often than expected from chance alone, and it supports the conclusion that some people are more susceptible to infection, or more often exposed to infection, than others.

No statistical analysis is required to demonstrate that there is no increase in the value for $\frac{de}{i(d + s)}$ with age, since the figures show that, in the age group over 20 years, the value is less than the value for all age groups combined.

APPENDIX 2.

RECOMMENDED SCALE OF FOOD RATIONS.

The following scale of food rationing is recommended by the Government of Southern Rhodesia. This scale of rations is not compulsory, but it is used by most employers if they employ large numbers of labourers. This scale is increased for a married man, and it is further increased for each dependent child.

Scale of Rations (per week):

Meal (maize, rice, or similar)	10½ lbs. (4.76 Kg.)
Meat (fresh, not more than 25% bone)	2½ lbs. (1.13 Kg.)
Beans (dried)	1 lb. (0.45 Kg.)
Salt	4 oz. (100 gm.)
Ground nuts, shelled	2 lbs. (0.9 Kg.)
Vegetables	2 lbs. (0.9 Kg.)
Potatoes or pumpkin	9 lbs. (4.1 Kg.)
Fish (as an alternative to meat)	2½ lbs. (1.13 Kg.)
Vegetable oil (if fish is supplied as an alternative to meat)	2 oz. (50 gm.)

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