The Fate of Schistosomes (S. Mansoni) in Experimental Infections of Normal and Vitamin A Deficient White Rats*

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IN EXPERIMENTAL infections of the white rat with cercariae of *S. mansoni*, the schistosomes mature and for the most part complete their life cycle in the liver. Only a few worms migrate to the larger mesenteric veins and rarely, if ever, do they reach the veins of the intestinal wall. Ova, apparently, are not passed in the feces, for frequent examinations of the excreta failed to demonstrate their presence.

In man, the normal host, schistosomes are found chiefly in portal and mesenteric veins, and in those of the intestinal wall; also, ova are passed in the excreta.

The reason for this essential difference between the induced and natural infections in rat and man is not at all clear. It may in part be due to the smallness of the mesenteric veins or to factors of a physico-chemical nature which inhibit such migration towards the intestine. To what extent this forced permanency in the liver is detrimental to the parasites will be discussed subsequently, and we also await further observations in larger experimental animals in which the parasites migrate to the mesenteric veins.

However, the results of our observations on the course of infection in the white rat seemed striking enough to merit detailed report. *Procedure*: White rats that have been inbred in this laboratory for many years were employed. When they were 28 days old, the animals were placed on a vitamin A free diet which consisted of:

Casein	18%
Starch	68%
Yeast	10%
Osborne and Mendel's salt mixture	4%
1 cc. viosterol per kilo of diet	

The infected control rats remained on a diet of Purina dog chow, which allows for excellent growth and fertility in stock animals.

* Received for publication March 14, 1940.

Each week the rats were weighed and their intake of food was recorded.

In order to compare the effects of schistosomal infection on the vitamin A free or deficient state, half the animals on the vitamin A free diet were infected when they began to lose weight, while the others served as non-infected controls. In four out of the six series of infected and control vitamin A free rats, no supplement of cod liver oil was given (Tables 1 to 4). In the last two series a single drop of cod liver oil was added once only (Tables 5 and 6). The ages at the time of infection of the animals on the normal diet ranged between 42 and 71 days and of those on the vitamin A free or deficient diet between 75 and 108 days, and in one instance, 131 days.

More or less equal numbers of vitamin A depleted rats and those on the normal diet were infected at the same time in the following manner:

To obtain a good yield of cercariae from infected snails of the species *Australorbis glabratus*, (*Planorbis guadeloupensis*) the following procedure is adopted: A fecal sample from a human patient containing large numbers of viable ova is broken up by stirring in water. It is then run through sieves of fine mesh into a large glass container where the material is allowed to sediment. The supernatant fluid is siphoned off and the sediment placed in a large tray with clean water to which several hundred snails are added. About five weeks later large numbers of cercariae are being passed daily by these snails. On the day of infection, at about 8:30 A.M., the infected snails are removed from their aquaria, rinsed and placed in two liter beakers almost filled with rain water. (The local tap water is frequently heavily charged with chlorine which exerts a deleterious effect upon both snails and cercariae.) These beakers are exposed to bright daylight. At 1:30 or 2:00 P.M. the daily cercarial output has reached its maximum. The snails are then removed. The contents are emptied into a clean beaker, except for the approximate last 100 cc. where are retained snail excreta and mucus and the less vigorous cercariae which have settled to the bottom. Any excreta or mucus transferred to the second beaker is readily removed with a pipette, leaving a pure suspension of cercariae.

The liquid is stirred with a glass rod. Apparent uniform distribution of cercariae is obtained when the movement of the liquid has almost subsided. Then a 1.5 ml. sample is removed by means of a pipette with a uniform inner diameter of 5 mm. The pipette is

calibrated each day of use by shifting the rubber bulb so that four samples constantly deliver 6 ml. of water when measured in a 10 ml. graduated cylinder. The sample with cercariae is discharged into a Syracuse watch glass with squares scratched on the lower surface after the manner of a blood counting chamber. The cercariae are killed by adding a few drops of concentrated hydrochloric acid and are then counted under a Greenough binocular microscope. Counts are made on two or three different samples and the average number of cercariae per ml. is calculated. One then estimates the number of mls. that will give the number of cercariae desired for infecting each animal. The calculated quantity of the cercarial suspension is measured into a 100 ml. graduated cylinder by means of a long glass tube of uniform width equipped with a large rubber bulb, and is stirred as before. The measured quantity in the cylinder is agitated to prevent adherence of cercariae to the sides and poured into a glass tumbler. One tumbler is prepared for each animal with an additional one chosen at random as a control. The cercariae in the latter are killed and counted as before to check the original estimate of cercariae. Later in the course of this investigation 50 ml. calibrated centrifuge tubes were substituted for the calibrated cylinder and the tumblers in order to eliminate one operation and to reduce loss of cercariae through adherence to the sides of the glass containers.

Each rat is placed in a jar containing enough rain water to reach the sides of the animal. The measured contents of the tumbler or centrifuge tube are shaken and poured into the jar. The rinsings of the tumbler or centrifuge tube are then added to the jar. This practically eliminates loss of adherent cercariae. The rats are left in the water about one hour.

It is realized that the number of cercariae to which an animal is exposed is, at best, an approximate one. When tumblers were used, the check count rarely disclosed an error 10 per cent greater or less than the estimated number of cercariae. With the centrifuge tubes this error was less than 5 per cent. The sources of error are (1) the adherence of live cercariae to the graduated pipette used in measuring 1.5 ml. for the first estimate and also to, (2) the large pipette used in transferring the cercariae from the beaker to the tumbler or centrifuge tube and, (3) the adherence of dead cercariae to the pipette and check container used in the final check count. By repeated checks of the number of adherent cercariae it is estimated that as many as three hundred may be lost in this way. (4) Variable numbers of cercariae never penetrate the mammalian host. It is

difficult to estimate the numbers lost in this way, as the water in which the rats have been placed becomes very turbid as a result of fecal and urinary contamination. Examination of less turbid samples leads one to believe that at times as much as 10 per cent of the cercariae may fail to penetrate.

As far as possible, infected animals and the vitamin A free or deficient controls were killed at definite intervals of time. The longest period an infected animal on the vitamin A free diet survived was 69 days after infection, and that with a supplement of a single drop of cod liver oil. Infected rats on the normal diet were allowed to survive for a maximum of 115 days after infection.

All animals were chloroformed and dissected at once. In the case of the infected animals, the following procedure was adopted in order to obtain all the schistosomes possible: To prevent loss of blood from the heart, liver and intestines, the inferior vena cava is tied a little above the renal level. Two additional ties are placed about the inferior vena cava immediately above the diaphragm and close to the right auricle of the heart, respectively. The portal vein is tied close to the hilus and again a little distal to that closer to the duodenum. A single ligature is placed about the distal portion of the colon to include the mesocolon and the main vein draining it. Smaller vessels about the cardiac end of the esophagus and gastro-hepatic ligament are also tied. By severing the supradiaphragmatic portion of the inferior vena cava between the two ties, the portal vein between its two ties and the inferior vena cava below the renal level, the whole of the liver can be removed with a portion of the diaphragm without loss of any blood. The liver is then weighed on balanced oiled paper, its external and sectioned surfaces examined and all blood lost from the liver during that procedure which has collected on the oiled paper is washed into a deep finger bowl containing normal saline. The liver is placed in the same bowl. The whole of the gastro-intestinal tract including spleen and pancreas is then lifted and removed en masse. This entails no loss of blood from the mesenteric veins. With a binocular loop the mesenteric veins are carefully examined either in bright sunlight or electric light and the number of worms is counted and their distribution noted. The spleen is then removed, and half of it saved in fixative; the other half is examined with a Greenough dissecting microscope. No worms or ova were ever identified within the spleen. Sections are taken from the mesentery, the pancreas, and gastro-intestinal tract after it has been opened and examined.

The blood from the heart is withdrawn with a pipette and collected in a Petri dish containing some normal saline. The pointed end of the pipette is passed into the superior vena cava and pulmonary artery, and as much blood as possible collected. The heart is then opened in situ and all blood clots removed and added to the dish.

Trachea, heart and lungs are removed together and placed in a Petri dish with some saline. The heart is weighed and placed in fixative. The trachea is severed a little above the bifurcation. The lungs are weighed and sectioned in the dish containing the saline. Small portions of two or three lobes are removed and placed in fixative. The remainder of the lung and fluid are kept for subsequent examination with the dissecting microscope.

All blood that has collected in the peritoneal and thoracic cavities as a result chiefly of the severing of the lowermost portions of the inferior vena cava and upper portion of the superior vena cava, is removed and placed in a Petri dish with some saline. The cavities are subsequently washed with saline and the washings added to the blood.

The rest of the dissection is carried out in the ordinary manner. The kidneys are weighed and portions put aside, occasionally together with fragments of other organs for examination with the dissecting microscope. Representative sections of all the other organs are saved in fixative, either Zenker or Formol-Zenker, and are subsequently sectioned and stained. Inasmuch as no sections of liver were taken in these series, an additional series of animals on normal and vitamin A free diets was infected and killed at corresponding intervals of time. Numerous sections of liver as well as other organs were fixed both in Zenker's fluid and 10 per cent formalin, and were sectioned and stained with hematoxylin-eosin and, at times, with various other stains.

In order to determine the numbers of live and dead schistosomes in liver, blood and various organs reserved for examination in the fresh state, a practicable method was developed by one of us (W. A. H.). Perfusion of the liver and lungs failed, because a large proportion of the parasites could not be expelled, especially those in small vessels. Nor could perfusion succeed in demonstrating destroyed or dying schistosomes, because these are almost invariably fixed in situ by reaction about them. This problem was solved by a modification of the pressed sample method employed in routine examination of hog muscle for *Trichinella spiralis*. The liver, in which the great

majority of schistosomes are found, is removed from the finger bowl and placed in a large culture dish. It is cut into several pieces and pressed with a square piece of thick glass. A small quantity of normal saline is added. This serves to release many flukes from the tissue. The freed parasites are removed with a pipette to a watch glass containing saline solution. The pressed fragments of liver are then further subdivided and dropped into a clean urinary sedimentation glass. Saline solution is added to the culture dish and its contents poured into the sedimentation glass. The culture dish is rinsed several times to remove all parasites and particles of liver. The rinsings are likewise added to the sedimentation glass. Each small piece of liver is then placed between two clean lantern slides and examined with a Greenough binocular microscope (40 mm. objectives, 10 x oculars). Illumination from a Spencer universal light transmitted through the tissue by means of the concave mirror proved sufficiently intense to provide good detail. Constant manual pressure on the lantern slides affords an even, thin field. By moving the slides in the manner of a mechanical stage, complete examination of all parts of the field is obtained. After all pieces of liver have been examined, the sediment in the urinary glass is pipetted on lantern slides and examined in the same way. As all flukes and small particles of tissue sink to the bottom it is not necessary to examine the supernatant fluid. When convenient, the flukes free in the sediment are added to those in the watch glass. The contents of the finger bowl in which the liver has been placed, lungs, spleen, occasionally other organs, heart's blood, thoracic and peritoneal washings and the contents of the other receptacles are all carefully examined. Solid organs and blood clot are treated as above. Fluids are examined either directly or removed to single lantern slides and examined under the microscope. Even the ligatures about blood vessels to which schistosomes frequently adhere are examined by direct overhead illumination. The parasites in heart's blood and lung are enumerated separately, and none saved. But all free ones from other sources are added to the watch glass and fixed in hot saturated bichloride and 2 per cent acetic acid overnight, and preserved in 70 per cent alcohol for enumeration and study.

Schistosomes emerging from the liver after sectioning and pressing and those in the extrahepatic portal vessels and heart are almost invariably alive. Those which remain in the liver may be dead or exhibit varied degrees of viability. As far as the lung is concerned, most have been found to be dead. The normally live schistosomes

in the tissues are usually fairly active. They possess a clear whitish cuticle sharply outlined from the surrounding tissues with no fixation at any point. The cecal contents are usually blackish and frequently are moved to and fro by muscular action of the gut. Transitional stages occur in which movements of the parasites and their cecal contents can barely be discerned and there are varied degrees of fixation of the parasites, partial or complete, by the tissues of the host. The typical dead schistosome is surrounded by a relatively thick bluish gray area of reaction in contradistinction to the greenish gray about the ova. There is loss of the cuticular outline and it may present a ragged, digested appearance. The substance of the parasite assumes a slate gray opaque appearance. The cecal contents are usually brown instead of black but their disposition in cecal pattern is maintained. Progressive degenerative changes of the parasite take place until little more than cecal pigment is identifiable. Lumped parasitic pigment alone is not included in the count of dead parasites unless in conjunction with some schistosomal structure. It is unlikely that any parasites have been missed by virtue of their blending with the surrounding tissues, for in the examination of thousands of schistosomes none have been found without pigmented cecal contents. Schistosomes can therefore readily be detected by this means.

This method possesses some disadvantages. Pressed preparations do not lend themselves to photography because of their thickness and inclusion between lantern slides. After subjection to pressure these preparations are of no value for paraffin sections. Yet, while this procedure cannot displace the use of sectioned material, it amply supplements it for, though sections show a great deal, unless cut serially they frequently present chance findings which may not reveal the true picture. In spite of the fact that pressed preparations lack the detail of sections, they show a great deal more than gross examinations. Insofar as small experimental animals are concerned, a fairly accurate census of the schistosomes present is provided. It is believed that the method may to some extent serve the purposes of anatomists and pathologists.

The number of animals employed was too small to permit one to judge whether the infection with Schistosoma mansoni in any way affected the course or character of the vitamin A free or deficient state as compared with uninfected controls. No significant difference in weight curves or duration of life was noted between the infected and non-infected animals on the vitamin A free or deficient diet, neither were the gross anatomical findings significantly different.

In earlier series in which lighter cercarial infections were employed more or less similar results were obtained, but were not as striking as those with heavier infections reported here. The results of these earlier series are accordingly omitted.

It can readily be seen from the tables (1–6) that quite different results were obtained in the two sets of animals. In those on the normal diet destruction of the parasites began almost as soon as they reached the liver and progressively became more pronounced, reaching its maximum between the fifth and seventh weeks. Thereafter, in general, there was a trend towards progressive reduction in the number of live worms with a minimum of 16 viable worms in two instances: Rat No. 153 on the 85th day and Rat No. 166 on the 115th day. The number of estimated dead worms also decreased, chiefly as a result of progressive resorption of lesions occasioned by their destruction and also on account of the difficulty of distinguishing the number of destroyed worms in a zone of reaction where (particularly in the later stages) quite a number might be destroyed in the same sector of vessel. There was only one exception to this general trend, and that was in Rat No. 119, where on the 29th day after infection there were only 4 dead out of a total of 401 worms.

By contrast, in the animals on the vitamin A free diet whether supplemented or not, there was with one exception either complete absence of, or at least relatively few, dead or destroyed parasites, up to as late as the 42nd day after infection. In Rat No. 145, whose diet was supplemented with one drop of cod liver oil 18 days before being killed, there were 66 dead parasites out of a total of 824 on the 42nd day after infection. Of the rats whose diet had been supplemented once and survived beyond the 42nd day, Rat No. 151 on the 57th day yielded an estimated 29 dead out of a total of 710 worms. In Rat No. 155, on the 63rd day, there were an estimated 100 dead in the liver and 273 live worms, but there was an estimated number of 124 in the lungs, many of which were probably dead. In Rat No. 157, on the 69th day, there were 70 dead out of a total of 622. So that even in these later stages the destruction of parasites is still quite reduced.

This difference between the two groups of rats on the different diets was so marked that had one not known the type of diet, one could readily predict from the number of parasites alive and dead to which group an animal belonged.

Owing to the rapid and massive destruction of the worms in the animals on the normal diet the number of live parasites is almost

always considerably greater in the vitamin A deficient rats, which might give one the impression that actually more worms reach and mature in the livers of these animals. It is difficult to arrive at an estimate of the number of dead and destroyed parasites because (1) in the early stages the young schistosomes are rapidly autolyzed with relatively little reaction and little pigment deposition; (2) even given a definite zone of reaction with parasitic pigmentation or cecal outlines, the actual number of parasites enclosed in this zone is very difficult to determine, and (3), relatively rapid resolution of the lesions of destroyed worms occurs. Despite this, if one determines the percentage of the estimated total number of worms (including live and dead) to the number of cercariae to which the animals were exposed, the following interesting figures are obtained. In the vitamin A free or deficient animals a maximum of 24 per cent and a minimum of 5.5 per cent are obtained. In those on the normal diet the maximum is 15.8 per cent twenty-four days after infection and the minimum 1.19 per cent, one hundred and eight days after infection. If one takes the average of all percentages in the first 42 days of the experiment (when the estimate of the number of those dead is a little more accurate), for those on the normal diet the average is 11.09 per cent, and for those on the vitamin A free or deficient diet, 11.33 per cent. This would imply that on the average about equal numbers of cercariae (11 per cent of their total) reach and mature in the liver on either diet. At very best, in these experiments 24 per cent of the total number of cercariae reached the liver. Failure of the cercariae to penetrate the skin, their destruction in the skin and along the complicated pathways before the liver is reached, account for this relatively low percentage.

Another very interesting observation is the degree of migration of the parasites away from the liver. The larger number of live worms found in the vitamin A deficient animals in the later stages of infection, permits of correspondingly larger numbers of worms in extrahepatic sites.* Thus, in the blood of the right side of the heart, eight were found in one vitamin A deficient rat (No. 151) on the 47th day after infection, forty in a second (No. 157) on the 69th day and sixteen in a third (No. 155) on the 63rd day. While in ani-

* In spite of careful search no direct anastomoses between portal and hepatic veins could be identified in the liver microscopically. From evidence that we have obtained in schistosomal experiments in the guinea pig we are led to believe that this migration to heart and lungs is due to portal-systemic anastomoses which develop and enlarge concomitant with increased pressure in the portal system due to destruction of parasites and reaction about eggs in the portal veins of the liver. This will be described in later communications.

mals on the normal diet occasionally one or more parasites were found in the heart's blood, the maximum was only seven in one instance (Rat No. 156) on the 64th day.

In the lungs there were 146 in one vitamin A deficient rat (No. 157) on the 69th day after infection and 124 in Rat No. 155 on the 63rd day. In the late stages of infection in animals on the normal diet there were anywhere from 15 to 57 parasites counted in the lungs, many of the worms in the lung were probably dead. A further indication that the lungs are an unsuitable medium for the parasites is the paucity of ova.

Limited migration to the mesenteric veins occurs earlier in the course of infection and greater numbers are again found in the vitamin A free or deficient animals. The parasites were found chiefly in the main superior mesenteric vein draining from the ileocecal angle, and occasionally in the splenic vein. The maximum number of worms, about 85, was found in one vitamin A deficient rat (No. 157) on the 57th day.

Neither age at the time of infection nor sex of the animal appear to influence the findings in the two groups. The weights of the animals at the time of infection can not be correlated with the subsequent number of parasites that are found, i. e., it does not follow that the heavier animals had fewer parasites than the lighter ones, or vice versa.

There was considerable variation in the size of the flukes from the vitamin A deficient rats, particularly from those that were killed from the 21st to the 42nd days of infection. *With but two exceptions this trend was not noted in any of the parasites from normal rats.*

The anatomical changes in the liver both in gross and microscopically amply confirm the observations made above.

GROSS ANATOMY OF LIVERS OF INFECTED ANIMALS

Normal Diet: In the rats on the normal diet there are no gross lesions in the liver before the 24th day after infection. Between the 24th and 28th day there are recognizable but minimal lesions, consisting of some slaty gray pigmentation in marginal areas at times associated with marginal atrophy and a yellow spot or oblong area, the site of early lesions about destroyed worms. Sectioned aspects of the liver occasionally reveal lobular, fibrous accentuation and some scattered, discrete small yellowish or white lesions, or a rare irregular gray translucent zone of reaction about a larger portal vessel.

After the 28th day and on or about the 32nd day, there are generally quite marked changes. The external surfaces of the liver, particularly the ventral aspects of the left lobe and the right and left accessory lobes reveal extensive linear and broader stellate or polygonal depressions. The polygonal and stellate areas often interrupt the linear scars. These scars are slate colored or of gray cast associated with yellow spots, largely in the broader stellate or polygonal areas. In spite of their irregular course the linear furrows appear to represent lesions involving the greater length of the subcapsular divisions of the portal vein. It is to be recalled that mature worms can stretch for a considerable length in a vessel, and that one or more may be killed in the same vessel. In addition, the external surfaces, particularly the ventral aspects of the left lobe are characterized by deep, often pigmented pits and lobular hypertrophy, lending to the surface a finely cobbled appearance. The marginal areas are extensively involved. Either they are wedge-shaped, or more continuous, involving a shorter or longer stretch of margin. Such confluent lesions are most often noted in the left lobe and along the sinistral border of the left accessory lobe. These marginal lesions are characterized by atrophy and thinning, a variable degree of pigmentation lending a fawnish-gray or darker slate colored appearance with orange colored spotting or stippling, and many coarse yellow areas. These are either round, oblong or of irregular shape and are either marginal or somewhat submarginal. Occasionally a single yellow modular lesion without surrounding changes or a marginal telangiectatic vessel may be seen on the dorsal aspect adjoining the marginal lesions where the liver substance presents a rippled effect. Sectioned surfaces of the liver reveal yellow subcapsular and deeper lesions either triangular or linear, often surrounded by gray infiltrated zones. It is to be emphasized that while subcapsular and marginal lesions are readily apparent externally, lending the impression that much of the destruction is at the extreme periphery, many deeper, more centralized lesions are seen on section. The marginal areas on section may merely reveal evidences of atrophic thinning with or without a grayish pigmented cast, or where yellow lesions occur, the marginal area may be transformed into homogeneous, gray-yellow tissue. Occasionally gray-white striae may be seen radiating at right angles from a prominent submarginal blood vessel. In these earlier stages many worms disgorge from the larger sectioned portal veins. The lobes of the liver most constantly and heavily involved are the left, left and right

accessory and papilliform lobes. The right and caudate lobes are generally only mildly affected.

From the 42nd day after infection to the end of the experimental period evidences of oviposition and progressive regression of lesions are noted. The external surface of the liver becomes smooth, as the cobbled effect in great part disappears with regression of the previous lobular parenchymatous hypertrophy. The submarginal rippling may persist in the late stages. There is, however, evidence of increased pigmentation. The interrupted, linear, gray lobular outlines often assume a slaty appearance. The more superficial linear furrows disappear while the deeper ones may persist, but are now more superficial and smaller. The yellow lesions indicative of active destruction of parasites, particularly in the broader expansions of the scars, disappear, and there may be either a large whitish spot denoting an organized sclerosed lesion of previous parasitic destruction or very small pin point white spots (measled effect) usually representing deposited ova with surrounding reaction. The marginal lesions, too, undergo changes. The thinned margins become thicker with occasional deeper notches, although there may be an abrupt transition to the thick portions of the subjacent liver, though with marked reduction in the number of living worms in later stages, many previous marginal lesions are almost completely restored to normal appearances. The still active, or recently active, marginal lesions where viable worms or recently destroyed worms are present are quite heavily pigmented, at times quite blackish, associated with a reticulated gray blackish capsular pattern with intervening red regenerated islands of liver tissue. A rare yellow nodule may be seen but there are more often larger whitish spots or gray-white streaks of older organizing and organized lesions. In addition there may be few or many pin point white spots of oviposition. The latter may also be found in the adjacent, more normal liver tissue, and a few here and there elsewhere in areas not definitely scarred. Terminal marginal vessels as seen by transmitted light are wide and sheathed in pigmented dense tissue. Rarely a small dark red varix is seen from which worms may disgorge.

The sectioned aspects of the liver in these later stages reveal at times fibrous lobular accentuation and scattered small white granules or gray white linear lesions. There is lack of general pigmentation as seen in the later stages of the vitamin A deficient animals but occasionally some perivenous areas of pigmentation are seen. Both marginal lesions and the infrequent external scars present on section

a more homogeneous gray appearance occasionally of darker pigmented cast with more discrete white spots or striae. At times there is a raised, green mosaic pattern enclosing reddish lacunar areas representing ectatic vessels about organized sectors of the portal vein. Very few worms escape from the larger sectioned vessels.

Vitamin A Deficient Diet: By contrast, in the vitamin A deficient animals the livers present no lesions in the first four weeks, except that there may or may not be some apparent pigmentation. After the 4th week and up to the 6th week there are often broad, smooth appearing, external areas of mahogany color, associated often with blackish lobular reticulation, particularly dorsally. There may or may not be some few scattered, small, yellow, roundish lesions. This is more apt to be seen in the supplemented animals. Except for some rippling marginally, there is otherwise no evidence of scarring, pitting or cobbled effect. Sections of the liver reveal in those areas underlying the mahogany zones fairly sharply outlined wide areas of gray pigmentation. There is portal accentuation. Lesions are scant, but there may be a few yellow or white spotted areas or small white nodules, particularly marginal, representing live parasites that are packed in dilated portal veins. In all instances numerous worms disgorge from the sectioned portal veins. In the 6th week oviposition makes itself manifest by many small white spots, and on section, in addition to general portal accentuation, fibrous vertical white lines and small white granular spots may be encountered. There may also be quite marked marginal clearing as seen by transmitted light, and outlines of worms packed in portal vessels may be discerned. In the 7th and 8th weeks the lesions are largely marginal consisting of nodular areas, either small or quite large, blocked or square, yellow or white, with considerable marginal pigmentation and measled effect. The atrophy, fibrous reticulation and irregularity of margin seen in the livers of animals on normal diets are practically entirely lacking. The large coarse nodules in particular represent massing and partial destruction of worms in the terminal portal veins, with exaggerated fibroblastic proliferation. The smaller nodular lesions represent either the occasional destroyed worm or the same exaggerated fibroblastic reaction about groups of ova. Sections of the liver at this stage reveal almost generally either a dirty gray, brownish pigmented or almost slaty black cast, associated with coarse gray reticulation and many small white spots. In addition there may be some yellow areas with surrounding gray zones. Fewer worms disgorge from the sectioned vessels.

Even as in the control vitamin A deficient animals, the common bile duct is often markedly widened and filled with yellow, soft, roundish or more flocculent material, with at times marked edematous thickening and vascularity of the parietes which involve the surrounding pancreatic tissue as well.

MICROSCOPIC ANATOMY OF LIVERS OF INFECTED ANIMALS

Normal Diet: On the 18th day there is irregular portal cellular infiltration chiefly involving smaller divisions and largely of eosinophilic character. Larger portal spaces reveal little cellular infiltration but apparently some edema. Inasmuch as in these early stages the parasites migrate readily towards the sectioned surfaces and the immersing or fixing fluid, few worms are seen in portal veins. Two parasites were found wedged in sinusoidal veins. One gains the impression that at this stage such attempted migration through sinusoidal vessels leads to rupture of the sinusoid, an area of microscopic hemorrhage and finally destruction and rapid absorption of the parasite, leaving some infiltrating round cells, heterophile polymorphonuclears, eosinophils and necrotic liver cells in its place. Such areas without recognizable parasites are not infrequently noted. There are too, rare focal parenchymatous necroses with heterophile polymorphonuclear infiltration. There is little evidence of parasitic pigmentation. Except for an occasional mitosis consistent with that found in normal livers, there is little evidence of proliferative activity. Kupffer cells are not particularly enlarged nor increased in numbers. Hence at this stage the small immature worms migrate probably widely throughout the liver concomitant with which there is early portal response with eosinophilic infiltration and slight evidence of parasitic destruction. The small size of the parasite at this stage precludes its fixation in the larger peripheral vessels although it is likely to be destroyed if it wanders into a sinusoid.

On the 28th day we note greater regularity in distribution of eosinophils in portal spaces which is quite intense about the medium sized vessels, slight about the very small ones and mild to moderate about the very large ones. Cross or tangential sections of intravascular parasites (chiefly of medium sized portal veins) are more frequently encountered as the increased size of the parasites and the thickening of the vascular walls tend to limit their migration. Many worms in the larger portal vessels do escape quite readily, however. It should perhaps be noted here that the peripheral portal

vessels as they enlarge, or with destruction of parasites where their collaterals enlarge, continue to receive parasites from the main vessels. The numbers of parasites in the larger vessels thus decrease as more migrate peripherally where they are progressively destroyed. In many instances there is marked endothelial hypertrophy of the involved vessels, which assume at times an almost columnar appearance. Mild or marked subendothelial eosinophilic infiltration is also observed. Lesions indicative of parasitic destruction are evident. These consist of intravascular infiltrations of eosinophils with few heterophiles and occasionally a strip of cuticle, indicating the remains of the parasite which had undergone rapid autolysis and heterolysis. The cuticle here, as in the later stages, is the more indigestible portion of the worm. Collateral portal vessels* in such areas are at times thrombosed and stuffed with eosinophils. The vascular walls are in great part obscured and overrun by eosinophils. In other instances the destructive parasitic lesions are essentially proliferative and made up of young fibroblasts with monocytic and giant cell reaction about cuticular remnants. Concomitant with these lesions there are not infrequent mid-zonal and central hyaline necroses or areas where the hepatic cells have in great part disappeared (autolyzed), leaving a vascular mesh with eosinophilic and round-celled infiltration. The margins, particularly those adjacent to destroyed parasites, reveal either atrophy or entire loss of parenchyma with cellular infiltration (chiefly eosinophilic) and reticular condensation. Elsewhere, there are subcapsular foci of monocytic, round cell and eosinophilic infiltration with or without early fibrosis. There is some infiltration about central lobular and hepatic veins. There is still a minimal amount of demonstrable parasitic pigment largely confined to monocytes within portal areas or in Kupffer cells towards the periphery of the lobules, or more heavily in the early subcapsular or marginal scars. Regeneration and proliferation of liver cells are as yet slight.

On the 35th day there is evidence of massive parasitic destruction. This is again characterized either by a thrombo-exudative, or more frequently, a proliferative phase of reaction. The latter may either represent a later stage of a preceding exudative phase or a local phenomenon in the course of the vessel which the parasite occupies.

* The term collateral portal vessels is used for convenience to denote widened vascular channels about the central portal vein which represent the anastomoses between portal vein and hepatic arterial plexus within the portal space. These are generally quite inconspicuous in the normal rat's liver.

From observations with fresh liver examined under the dissecting microscope it is evident that the worm as a whole may not be destroyed simultaneously, but that only a part of it may be affected. It may be constricted within the vessel at one point whereas the rest may still be free and well preserved. In other words, while there may be a sharp eosinophilic thrombotic reaction about one sector of the worm, other sectors undergo a slower death with hyalinization which may be followed almost simultaneously by a proliferative response of fibroblasts and monocytes. The thrombo-exudative reaction, on the other hand, does not only follow the death of the parasite. Fixation of the parasite in the portal vessel excites a greater perivascular cellular response which may extend beyond the limits of the endothelium producing an intravascular thrombus. This completely immures the parasite within the vessel. Furthermore there is a greater tendency for more than one worm to be destroyed in the same vascular segment. Thus, occasionally in the length of a long widened peripheral portal vein there may be distally necrotic worms and proximally, viable appearing ones. These are separated either by a regenerated endothelial shelf or by a fibrinous thrombus which tends to enclose the viable worms. Once destroyed, the segment of necrotic worm is completely overrun, chiefly by eosinophilic cells. Cuticular and subcuticular portions only remain and about these, large pale vacuolated histiocytes and multinucleated giant cells gather, and peripherally monocytes and fibroblasts proliferate to a greater or lesser degree. Even in the more exclusively proliferative lesions, necrotic eosinophils are often grouped centrally and in the more exudative lesions there are quite marked necrosis and disintegration of the eosinophils with accompanying phagocytosis of their remains by ambient monocytes. At times these reactive changes are confined to greatly widened, thickened and cellular portal spaces; at other times, particularly in small divisions, wider or narrower zones of surrounding liver tissue share in the reaction either with atrophy, partly as a result of compression due to the rapid cellular infiltration of the portal area, or by solid hyalinization and necrosis. Monocytes and fibroblasts or infiltration by eosinophils and some round cells occur in these areas between the atrophic or necrotic liver cells, or more solidly where these have disappeared. The reaction often extends to the external capsule. Bile ducts in the thickened portal areas lengthen. Their lining cells are large and pale and an occasional one is in mitosis.

In place chiefly of a single central portal vein in a portal space

there may be now wide collaterals. These often surround a central proliferative and organizing (or less frequently, an exudative) parasitic lesion. At times, particularly with the latter type of lesion, one or more of these collaterals are thrombosed and stuffed with eosinophils. The septa between these collaterals are thick and cellular, chiefly eosinophilic, even as in the rest of the portal space.

Even at this stage there are already evidences of organized lesions with no trace of the parasite. These appear as single or multiple nodules made up of young fibroblasts with little collagen, vacuolated monocytes containing fat, an occasional giant cell and often heavy parasitic pigment deposits. The types of parenchymatous necroses described under the 28th day liver are again found here with added small hemorrhages, sinusoids thrombosed both by fibrin and eosinophils and very active phagocytosis by Kupffer cells and histiocytes. These necroses in part are apparently due to rapid occlusion of the central portal vein by the reaction to the parasite as well as the thrombosed collaterals.

In addition, however, there are marked reparative and proliferative processes attested to by the numerous mitoses. Thus, active regeneration of liver cells about portal spaces that are involved in the destruction of parasites is clearly apparent. Proliferative processes not on the basis of regeneration are best seen subcapsularly where the recently proliferated large pale cells elevate the capsule giving it its raised lobulated or cobbled character. At the same time, portal and hepatic veins reaching the capsule tend to draw it inwardly by virtue of cellular infiltration and, in the latter instance, by increase in fibrillar connective tissue. Furthermore there are irregular fibrillar scars radiating from portal areas (generally the sites of parasitic destruction) towards central or hepatic veins. The scars as well as the hepatic veins are infiltrated by numbers of eosinophils, some round cells and pigment containing monocytes which at times also contain globules of fat. These scars sharply outline irregular lobules which reveal alteration in arrangement of cords and relationship to central veins.

Marginal areas are considerably atrophic, there being loss of liver tissue, reticular condensation and cellular infiltration correlated with parasitic lesions of submarginal terminal portal veins or the very marginal segments of these. Massive deposits of parasitic pigment occur in and about destroyed worms, with lighter deposits in the fibrillar scars, and hypertrophied Kupffer cells here and there adjoining the involved portal areas. There is marked fatty infiltration

of the cells involved in the proliferative parasitic reactions both in the monocytes and young fibroblasts.

On the 42nd day there are few changes in character or type of lesions from those observed on the 35th day. There are some recently deposited ova surrounded by giant cells or monocytes. The same cells may also be found within the deformed egg shell. Disintegration of eosinophils in portal and other infiltrated areas and in zones of organization of dead parasites are apparent and lymphocytes with some plasma cells are more frequent. Active destruction of worms is still in progress although there are now more frequent evidences of older lesions sharply bordered and better outlined, made up of small nodules of fibroblastic and monocytoid cells, one or both being stuffed with globules of fat and enclosing a fair abundance of parasitic pigment. The eosinophils originally within this zone of organization have disintegrated and disappeared and the fibroblasts have become reduced in number and size. These older lesions are either oval in cross section or cylindrical in tangential sections. There is in general greater concentration of parasitic pigment, but little evident stainable iron in adjoining Kupffer cells and radiating scars. The latter are thicker and more fibrillar. The lobules are larger, with more compact, regenerated, and more normally arranged, liver cells. Parasites which appear to be viable are squeezed within thick walled, probably hypertrophied, portal veins. More of these are seen both in portal veins apparently previously involved as well as in those where active organization of dead worms is proceeding. Occasionally a fibrinous thrombus separates the dead from the living.

In the 50th and 56th day periods there are very wide ectatic portal veins, particularly peripherally, either largely as a single central vessel in the portal space or multivascular and angiomatoid. Often in the latter there are many viable parasites, or in the former, more recent and earlier organized parasitic nodules. The earlier and more diffuse portal eosinophilic infiltration has in great part disappeared and in many sections there is little or no infiltration and the sections generally are practically normal. There is, however, fairly diffuse fibrous perilobular and pseudolobular scarring. The involved margins reveal rather extensive scarring or reticular condensation with marked regenerative lobulation of liver tissue in instances, or they remain essentially reticular with few scattered liver cells and infiltrating eosinophils and round cells. There are frequent ova, particularly marginally and peripherally near the

capsule or within thick portal zones bordering the ectatic veins. In the latter region they tend to be more frequent and are often massed, and thus add to the deformity of the vessels. These ova have been deposited by worms lying within the wide portal veins. As the ova are released they are rapidly excluded from the circulation by an endothelial covering much as any foreign body, inducing, however, at times eosinophilic thrombosis. The reaction about them is variable, at times associated with considerable fibroblastic proliferation, at other times merely associated with some monocytes or giant cells.

There are not infrequent recent hyaline necrotic worms. The reactions about them are strictly intravascular and largely proliferative.

The distribution of pigment is much as in the earlier stages, i.e., focal, localized to radiating scars, large, previously involved portal areas and adjoining Kupffer cells. This is due to the destruction of the parasites with consequent release of pigment in these areas. The more uniform distribution of parasitic pigment within Kupffer cells of the liver (as in human schistosomiasis) is lacking because of the few worms in portal and mesenteric vessels.

On and after the 63rd day regressive changes as noted in gross examination are now quite evident. Subcapsular lobular hypertrophy and the previous infiltration of hepatic veins which accounted for the pitting and cobbling of the surface have in great part disappeared, remaining only in marginal areas where it accounts for the rippled effect. Frequent sections of liver at this stage are practically normal except for portal areas here and there which are quite heavily pigmented and infiltrated by round cells with few eosinophils. Elsewhere there are occasional small fibroblastic pigmented nodules or hyalinized nodules of previous parasitic destruction with little collateral venous ectasis and with or without surrounding pseudolobulation or fibrosis. These portal areas are generally very lightly, if at all, infiltrated by round cells with very few eosinophils but are still quite thick, hyalinized and somewhat edematous. In contradistinction to areas where viable or recently destroyed worms are still present there is light pigmentation of either the portal area or the hyalinized, irregular, poorly-cellular surrounding scars. The pigment is of two types: parasitic and of the free iron type which takes the Prussia blue reaction. Often, therefore, within the same monocyte, parasitic and iron free pigment are demonstrable. There is little pigment in the Kupffer cells in these areas. This dilution of parasitic pigment implies either its progressive transportation

to other distant areas or organs, or (in part) its progressive local metabolic conversion questionably into an iron free hemosiderin-like substance. For both here and in the heavier pigmented regions where viable and recently destroyed parasites exist, there is marked increase in iron containing pigment in these later stages, probably not entirely accounted for by organization of thrombus or breakdown of red cells. As for these latter areas (generally more marginal) there is progressive decrease in the frequency with which one encounters wide portal veins with numbers of viable parasites. Hyalinized necrotic worms are quite frequent. Monocytic and giant cells are closely applied to these and about them there are concentric rings of fibroblasts with a thin or thicker reticulum about each. The sharp exudative phases of parasitic destruction of the earlier stages are entirely lacking. About the organizing area within the thick portal space, there may be more or less marked cellular infiltration, but chiefly lymphocytic. There may or may not be wide collateral vessels. About these there are often goodly scarring and reticular condensation extending to the capsule or to the very margin. This is generally associated with quite marked regenerative multilobulation of the liver. Whereas in the old organized and regressed zones of parasitic destruction, the surrounding scars become thinner and more delicate with only a rare eosinophil or round cell, in the areas of active destruction these are often still quite cellular and infiltrated both by round cells and eosinophils.

There are generally many ova either single or massed, either subcapsular and associated with a fairly wide irregular zone of more hyaline fibrosis, or marginal, or, more often, within the thickened portal areas with either wide patent or organized veins. The reactions about these eggs are much as described above and of variable character. Few of them appear to contain viable miracidia. The shells are often distorted and monocytes, giant cells, and occasionally some eosinophils are often within the egg shell where the inner cellular mass has been destroyed and phagocytosed. About the egg shells there may be little more than some flattened monocytes or multinucleated giant cells with surrounding round cells or, for some reason not entirely clear, quite marked fibroblastic reaction. In some instances, in addition, there is sharp, almost necrotizing, eosinophilic response to the presence of the eggs, which largely involves the inner cellular mass. In a few instances a fragment of the shell could be identified in a small poorly cellular and hyalinized

nodule representing the late stages of the reaction occasioned by the ovum.

Vitamin A deficient Diet: The livers of the vitamin A deficient group that were studied microscopically were taken from rats all of which had been supplemented once with a drop of cod liver oil. In those that were killed or died between the 18th and 32nd day after infection, the supplement had been given 5 to 9 days after infection. From the 35th to the 65th day, when the last animal in this group was killed, all had been supplemented on the 32nd day after infection.

There is practically no reaction to the presence of the parasites up to the 32nd day. Some portal thickening and edema is noted but there is no cellular infiltration except for some bile pigmented monocytes. In those rats that presented thickened, common bile ducts with yellow soft calculous particles within the widened lumen, there is also apparent bile duct proliferation extending irregularly into the adjoining lobules. But in many instances whether the common bile duct was or was not dilated, smaller biliary radicles often contained either a hyaline acidophilic material, or granular and crystalline deposits with occasionally frank bacterial colonies which might provoke, however, no inflammatory response. In the thickened common bile duct, on the other hand, there are often bacterial colonies with parietal necrosis, ulceration and acute and chronic inflammation.

In those animals that were killed, the parasites had largely migrated towards the fixing fluid. In those that died and were autopsied soon afterwards, there are numerous, somewhat immature-appearing parasites in the large and medium sized portal veins. There are none that are definitely dead or disintegrating.

The liver cells present often quite marked grades of atrophy with very few mitoses. The sinusoids are wide. The Kupffer cells are not particularly hypertrophied or active. There is very little apparent parasitic pigmentation.

On the 35th day the larger portal veins are packed with parasites. Endothelial hypertrophy and at times moderate, intimal thickening and cellular infiltration of these veins are noted. In general, however, there is slight portal infiltration by round cells or eosinophils although in instances this is quite marked. There is little evident destruction of parasites. There are either a rare collateral portal venous thrombus made up largely of eosinophils or a rare mural or more massive thrombus in the widened central portal vessel which

enclosed in one instance viable-appearing parasites. There are, too, rare small periportal or mid-zonal necroses. The Kupffer cells are somewhat hypertrophied and contain parasitic pigment.

On the 42nd day the portal spaces are exceptionally thick and prominent. This is in large part due to quite marked round-celled infiltration with but few eosinophils. In instances there is associated fibroblastic proliferation without demonstrable ova or dead worms. There is apparently quite marked proliferation of bile ducts and an occasional one contains bacterial colonies. There are frequent recently deposited eggs, particularly in smaller portal vessels. These are surrounded by round cells, some eosinophils and, in instances, by fibroblasts. The inner portions of some of the eggs are invaded by many eosinophils. The peripheral portal veins are frequently mildly dilated and occupied by one or more viable appearing worms. In a few instances these parasites present, however, early but frank growth of bacteria within their intestinal ceca. There is only a rare lesion (presumably of a dead and organized worm) characterized by concentric fibroblasts and some multinucleated giant cells. Moderate endothelial hypertrophy of the parasitized veins persists. As for the liver elsewhere, in spite of atrophy, there are numerous mitoses. Occasional mitoses are noted in the epithelium of bile ducts and of Kupffer cells. The latter are markedly hypertrophied, increased in number and present active phagocytosis of parasitic pigment, cellular débris and erythrocytes. There are frequent intrasinusoidal aggregates of either monocytes with some round cells or of heterophile polymorphonuclears. There are also rare parenchymatous necroses.

On the 49th day the outstanding features are the large fibroblastic nodules which occasionally project beyond the level of the external capsule. In the centers of these there are necrotic polymorphonuclears with, in instances, some recognizable remains of parasites. This reaction towards dead parasites is therefore characterized by excessive fibroblastic proliferation without the rapid regression noted in those on the normal diet, as well as by the lack of eosinophilic response and of dilatation of the collateral plexus of veins in the portal spaces. These nodules produce a degree of surrounding parenchymatous atrophy. In rare instances, in association with a dead parasite, the reaction is essentially thrombotic and more frankly of heterophile polymorphonuclear type. There are only rare lesions indicative of old and past destruction of parasites or ova such as a small fibrous pigmented nodule or a hyaline one with the remains of a worm centrally placed. In general there are fewer

changes than on the 42nd day, with practically no parenchymatous mitoses, little hyperplasia of Kupffer cells, less infiltration of portal spaces and little hyperplasia of the bile ducts.

On the 56th day there are similar proliferative fibroblastic nodules as on the 49th day, but in this instance such reaction not only occurs about destroyed parasites but about ova as well, or about both. These represent older reactions to the ova. The more recently deposited ones are dealt with by monocytes or giant cells with round-celled reaction or at times by heterophile polymorphonuclears which also invade the interior of the eggs. In other respects it resembles the liver of the 49th day with, however, frequent mitoses of liver cells. Both on the 49th and 56th days no frank growth of bacteria is identified in the ceca of viable parasites.

On the 65th day there are profound histological changes. The most outstanding feature is the amazing growth of relatively short, presumably Gram positive bacilli in the ceca of the parasites. Few parasites are spared. There are some which contain few, while in others the bacterial growth has produced great enlargement of the ceca, leaving only a shell of atrophic extracecal tissue. The bacterial growth is strictly intracecal and results in its distension and enlargement but without frank extracecal invasion. In advanced stages the parasite disintegrates and ruptures, liberating the mass of bacteria which are rapidly engulfed by the ensuing heterophile polymorphonuclear infiltration. This leads to frank small pylephlebitic abscesses.

The portal spaces are thick, though generally with moderate or light, round-celled infiltration, including many plasma cells, frequent heterophile polymorphonuclears and large, heavily pigmented monocytes. Rarely there is a hyalinized thickened portal space. There is marked apparent bile duct proliferation. Some of the bile ducts contain bacterial masses, hyaline débris and in a few instances heterophile polymorphonuclears. In addition to the pylephlebitic abscesses there are quite frequent thrombosed smaller portal veins containing fibrin, with or without polymorphonuclears, occasionally in association with a recently deposited egg. A single thrombosed hepatic vein with massed heterophile polymorphonuclears is seen. There are many recently deposited eggs but there are also older lesions of nodular fibroblastic type as in the 49th and 56th days, showing in all a little more regression with thicker reticulum and fewer fibroblasts, but these are more abundant and plumper than at any stage in the animals on a normal diet. There is marked atrophy

of the liver with frequent necrotic cells, but no mitoses. The sinusoids are exceedingly cellular. Aggregates of hypertrophied and proliferated Kupffer cells (with rare mitoses) are quite frequent. These are generally quite heavily pigmented and reveal active phagocytosis of red cells. Intrasinusoidal clusters of heterophile polymorphonuclears are also frequent and at times there are fibrinous thrombi. There are pigmented monocytes in the walls of the hepatic veins. The pigment here as in Kupffer cells and portal spaces is practically entirely of parasitic type. There is little demonstrable iron. This is also true of the earlier stages in the Vitamin A deficient series. There is also little demonstrable fat either in liver cells or proliferative nodules but more in occasional small clusters of large pale portal monocytes.

Of the other organs, the lungs are of particular interest. Few viable appearing worms are found in the lungs of the animals on the normal diet; many more, however, in those on the deficient diet. These lie either freely within the larger parahilar branches of the pulmonary artery or are crowded and stretched within widened and thinned peripheral ones. In the deficient animals where more parasites are seen there is little reaction primarily to their presence except for some endothelial hypertrophy and in the smaller vessels some perivascular lymphocytic infiltration. There are many lesions about dead and destroyed parasites. These are largely peripheral particularly subpleural and could be identified grossly by their raised gray character with central whiteish areas. The lesions all in all resemble those in the livers of the same animals and to a degree the same processes leading to the destruction of the parasites can be traced. Not infrequent viable-appearing worms present cecal bacterial overgrowth. This, however, never reaches the proportions seen in the liver. The parasites appear to die much sooner. Their death and destruction leads at first to intra-arterial abscesses, followed subsequently by exaggerated surrounding proliferative fibroblastic and histiocytic reaction with considerable lymphocytic infiltration. In other instances, with the destruction of one or more worms in one sector others are killed off as a result of the concomitant reaction or thrombosis. Occasionally the necrotic remains of the parasite have ulcerated into the lumen of a bronchiole.

There are at best but few ova in the lung and these produce but little reaction, which consists mainly of some monocytes and giant cells, except in the deficient animals where exaggerated monocytic and round-celled reaction is noted about the ova.

In the vitamin A deficient animals parasitic pigment in the spleen can be identified earlier and is generally more abundant, due to the greater number of live worms in the liver. In the late stages of infection in the animals on the normal diet the pigmentation, however, is quite marked. In gross, this pigmentation could best be identified within the lymphoid nodules where the normal white follicles assumed a slate-gray and even darker color. It was our experience that the pigment is chiefly concentrated within the reticular cells of the follicles (particularly their central portions) with but little in the heavily hemosiderin-laden monocytes of the pulp.

The mesenteric veins at no time present evidences of any reaction even when moderately heavily parasitized.

There is quite constant enlargement of the lymph nodes at the head end of the pancreas, adjoining the distal end of the common bile duct. These histologically present lymphoid hyperplasia, wide sinuses with many monocytes containing hemosiderin but no definitely identifiable parasitic pigment.

Sections of femoral bone marrow of all the series included in the experiment, and smears of the marrow stained by Wright's of all the animals employed for histological studies of the liver, were reviewed. No differential counts were made. It is quite apparent that in the animals on the normal diet the marrow is always richly cellular with maximum numbers of maturing and mature eosinophils from about the 5th to the 7th week after infection. The numbers of eosinophils then appear to decline to a more normal level. In the vitamin A deficient animals there is hypoplasia of the marrow with quite marked fatty replacement. There is no particular eosinophilia at any time.

DISCUSSION

If one attempts to reconstruct the sequence of events leading to the marked reduction in number of live worms in the rats on the normal diet it is perhaps best to recall two things. First, the liver is an unfavorable medium for the parasites, partly as a result of physical factors. The portal vein, except for its juxtahilar divisions, is essentially a system of narrow ramifying branches which come off more or less at right angles to the parent stem. In such system the parasites have not the freedom of movement or space as in the main portal or mesenteric veins. This likewise applies to the distal branches of the pulmonary artery in the lung. Second, there are the observations that were made during the examination of the fresh

livers. In the earlier stages of infection, worms freely escaped with the blood or actively worked their way out towards the immersing fluid from larger portal venous radicles when the liver was sectioned into small pieces. When these fragments of liver were crushed under a heavy glass slide more live worms were liberated. These in part represented parasites already tightly wedged in peripheral smaller portal veins but which could still be released by disrupting the surrounding tissue. In addition, there were other parasites still viable that were fixed in the vein either as a whole or in part, as revealed by examination with the dissecting microscope. As a general rule, therefore, it was found that as more time after infection elapsed, there were fewer free live worms that escaped spontaneously from larger sectioned portal veins and more were immobilized partly or completely in narrower peripheral veins concomitant with the increasing number of definitely dead and destroyed ones.

This was substantiated histologically. In the very early stages when the parasites were small and immature they were more uniformly distributed throughout the portal radicles of the liver, provoking a mild eosinophilic reaction. In later stages, on and after the 35th day after infection, the obviously live and normal worms were packed within the portal vein at the hilus and its larger radicles. In these wide channels there was little perivenous reaction and it was very unusual for nodules of destroyed worms to be identified. The destructive process, however, was centered in the peripheral vessels, those in marginal, submarginal and subcapsular sites. Some of these veins widened to accommodate the worms which have been forced centrifugally for unknown reasons away from their natural routes of migration to the mesenteric veins. The reaction about such veins might be slight. In others, on the other hand, whether moderately widened or not, there was intense eosinophilic and edematous parietal and subendothelial infiltration which served to narrow the lumen and to inhibit the movements of the parasite. It was not infrequent that under such circumstances a small thrombus of eosinophilic cells was noted to one side of the vessel particularly where the main vein of the portal space communicated with one of the vessels of the surrounding widened collateral plexus, or at its bifurcation. This further helped to immobilize a part of the worm in the vein. It was also felt that the apparently hypertrophied musculature of the peripheral portal vein even as that of the small pulmonary arteries might by contraction either hinder the movements of the parasite or force it more distally. The conviction, therefore, grew that as

long as the parasite was active within a large vein it was not likely to be destroyed; but once it wandered into narrow peripheral vessels where it could move less freely, it was likely to be partly or completely immobilized in the vessel as a result chiefly of the sharp perivascular and subendothelial reaction (often with mural thrombi) induced by its close and more prolonged contact with the vascular wall. Henceforth the immobilized parasite acted as a complex organic foreign body towards which the host reacted in an augmented manner both preceding and following its death. There were enhanced perivascular cellular infiltration and intravascular reaction and thrombosis sufficient at times to squeeze and distort the parasite. Invasion and digestion of the body of the parasite by the cells of the host (eosinophils chiefly) could occur even before its death. Hence the death of the parasite could be due to the cutting off of blood flow as a result of thrombosis, forcible compression, and direct invasion and digestion of the parasite by the cells of the host. Sectors of the same worm, not immobilized but projecting free into another part of the vessel, apparently underwent subsequent hyaline changes and were disposed of by a slower proliferative process of organization.

In the late stages of infection after considerable parasitic destruction has occurred the number of worms free in the large portal venous radicles is considerably reduced. As a result, either greatly widened or at times varicosed and often canalized main veins of peripheral portal spaces (where worms had previously been destroyed) or those of the surrounding collateral plexus accommodated more live worms; these were often of adequate width to permit the worms to move about freely. Constant deposition of eggs in these vessels with their subsequent parietal organization produced shelves and tongue-like projections into the vascular lumen. Additional worms could therefore be destroyed by one of several mechanisms: (1) the wedging of the parasite within such points of narrowing in the vessel, (2) the fixation of the parasite secondary to a thrombus set up by ova or the worm itself, or (3) the enclosure of the worm within a thrombus adjoining a more recently destroyed parasite. This last mechanism was also frequently operative in earlier stages.

It seems therefore that the chief mechanism of destruction of the schistosomes in the rat on the normal diet is due primarily to the migration of the parasites into narrow blood vessels such as the peripheral branches of the portal vein or the small arteries of the lung. If these are capable of widening to a degree which will permit the parasites to move freely within them, without close parietal

contact, then it is unlikely that the worms will be destroyed except for reasons like that of oviposition described above. Usually, however, these vessels are incapable of enlarging to any extent, particularly in earlier stages of infection and the close and continued contact of their parietes with the parasites induces sharp reactive changes which serve to immobilize, fix and subsequently to destroy the worms. There was no evidence that any precipitin immune effect either about cuticle, oral parts or in the ceca occurred in the way described by Sarles and Taliaferro[1] and Sarles[2] in the case of *Nippostrongylus muris*.

In the rats on the vitamin A free or deficient diet where there was practically no destruction of the parasites in the first 35 or 42 days after infection there were by contrast, (1) almost complete lack of any cellular reaction about parasitized portal veins; (2) greater ability of the peripheral portal veins to dilate, partly as a result of cause (1), and partly as a result of parenchymatous atrophy due to the vitamin A deficient state; and (3) greater variations in the size of the parasites, many remaining quite small and immature. Hence there was less continued close contact between parasites and the walls of the blood vessels and strikingly less reaction on the part of the host.

From the 42nd day onwards whatever destruction of parasites did occur was due to a twofold mechanism. One of these concerned the changes in the portal spaces. Constant parasitization as well as some added reactive ability on the part of the host as a result of the cod liver oil supplement were not without its effect on the portal veins. These thickened and were infiltrated chiefly by lymphocytes. Although in the control vitamin A deficient rats there was a certain amount of biliary cirrhosis, i.e., apparent bile duct proliferation and lymphocytic infiltration chiefly secondary to the ulcerative choledochitis, it generally did not reach the proportions seen in the infected animals. In addition, heavy deposits of ova, with the exaggerated fibroblastic reaction about them, served to narrow the vascular lumen. As a result, therefore, either of marked narrowing of the vessel at one or more points or thrombosis set up by oviposition or irritation induced by the parasite, certain numbers of worms were thus destroyed. The second mechanism was one in which the worms were killed as a result of bacterial invasion and growth in their ceca. This at times assumed marked proportions in which the ceca were distended forming huge cysts filled with bacteria. These

subsequently ruptured, liberating the mass of bacteria, inducing pylephlebitic or intra-arterial pulmonary abscesses. This bacterial invasion of the ceca of the parasites can be accounted for by the greater bacterial permeability of the intestine as shown by Verder[3] and Lassen[4] and by the increased bacterial content of the intestine by Cramer[5] and Seidmon and Arnold[6] in vitamin A deficient animals.

Inasmuch as this invasion apparently only occurred after the 6th week of infection it may be that it was dependent upon several factors: (1) the degree of maturation of the parasite; (2) the degree of bacteremia in the host; (3) the type of micro-organism; (4) the effect of the vitamin A deficiency on the parasite itself or the outcome of its own depletion of the vitamin A factor; (5) disturbances in the contractile ability of the ceca of the parasites, perhaps depending on cause (4), leading to a paresis or paralysis, and creating thereby a stagnant medium; (6) some change in the pH reaction of the ceca which would permit the growth of bacteria.

As far as one can tell at present the bacteria in the ceca of the parasites appear to be Gram positive bacilli. They may, therefore, be *Lactobacillus acidophilus*, the common organism of the bacterial flora of the intestines of rats. Their acid resisting properties might be particularly suited for their growth in the parasitic ceca if these should prove to be acid. Certainly the rapid conversion of red blood cells into a dark brown pigment within the ceca would attest to their considerable degree of acidity or alkalinity.

This study has served to shed some light on the differences of reaction towards a helminthological infection by a host on a vitamin A deficient diet as compared with one on a normal diet. In addition to the lack of eosinophilic and cellular response to the parasitization of portal veins in the earlier stages of the infection in vitamin A deficient animals there were also quite marked differences in later stages in the way destroyed parasites and ova were handled. In the animals on the normal diet, particularly about the 5th and 6th weeks when destruction of the parasites was maximum, reaction and repair were sharp and rapid. There was either an intravascular eosinophilic thrombus with active disintegration of the parasite by the invading eosinophils or the reaction was to a greater extent proliferative. In the latter instance, monocytes or epithelioid cells with the formation of giant cells played the more important rôle in digesting cuticle and necrotic hyaline sectors of the worms. Associated with these sharp changes there were quite marked surrounding or more distant lobular necroses with or without hemorrhage and with

eosinophilic and round-celled infiltration. At the same time, or a little later, the sites of destroyed parasites were converted into small fibrous nodules or cylinders with some remaining large monocytes stuffed with parasitic and iron pigment. There were active regeneration and beyond that excessive proliferation of liver tissue leading to multilobulation about delicate fibrillar scars; and subcapsular hypertrophy lending the cobbled effect to the external surface of the liver. In brief time even these changes regressed in great part, and reactive changes persisted only in areas where viable parasites remained and where oviposition had taken place. Destroyed parasites at this stage were dealt with by slower organization performed by monocytes with a surrounding border of rather thick collagenous but fairly cellular fibrous tissue. There was little evidence of surrounding parenchymatous reaction or the sharp eosinophilic response of the earlier stages.

In the vitamin A deficient animals, however, besides the frank abscesses caused by bacterial invasion of the parasites, where death was due to some other mechanism, their organization was characterized by its excessive fibroblastic proliferation. Fibroblasts grew throughout the thick prominent portal space and extended inwardly to surround the dead parasite. It was accompanied by a variable amount of lymphocytic, heterophile polymorphonuclear, eosinophilic and monocytic reaction but remained in general chiefly fibroblastic. These reactive areas formed largish nodules which were quite distinctive in the gross examination of the liver. Furthermore, in addition to this excessive fibroblastic proliferation there was marked retardation in the regression of the lesion even where the parasite had to all appearances been completely digested, i.e., the reduction in the number and size of fibroblasts and the deposition of more collagen were retarded. Precisely the same response was observed in the lungs about dead parasites. About ova the same differences were noted. Here too there was excessive fibroblastic proliferation with retarded regression as compared with that seen in animals on the normal diet.

Contrariwise, there was at best only mild or moderate regeneration of liver cells, but little evident excessive proliferation in the vitamin A deficient animals as compared with those on a normal diet, i.e., the vitamin A deficient state permitted of excessive proliferation of fibroblasts but retarded the proliferation of more highly organized cells such as those of the parenchyma of the liver.

There was no evidence that phagocytosis was in any way hindered

in infected vitamin A deficient animals. It is frequently stated in the literature that there is injury to, or lack of response of, the reticulo-endothelial system in the vitamin A deficient state. In fact, here one was impressed by the pronounced activity of the Kupffer cells of the liver, by their hypertrophy and proliferation; and even in the spleen, although there was considerable atrophy of lymphoid tissue, the reticular cells of the follicles and the monocytes of the pulp were actively phagocytic.

In the excellent reviews on the subject of the effects of nutrition and vitamins upon the resistance of the host to infection, Clausen[7] and Robertson[8] observed that in the vitamin A deficient state there was little evidence to indicate either a loss of circulating antibodies or a failure of their production, although they might be quantitatively reduced. It is realized that here we are dealing with an abnormal host, but the conclusions to be drawn from the results of these experiments seem to be applicable in general to helminthological problems of resistance or immunity at least as far as dietary conditions are concerned. Hence, in those helminthological infections where tissue immunity appears to play the greater róle, the effects of a normal as compared with a deficient diet serve to demonstrate from an anatomical viewpoint precisely how the greater resistance of the host on the normal diet (implying thereby decreased survival rate of the parasites) is brought about. To us this seems to be dependent upon several essential factors: (1) the degree of contact of the parasite with the tissues of the host, i.e., there will be less contact of parasite with the wall of larger blood vessels and ducts (such as common bile duct or bronchus) than in smaller ones and little contact in the lumen of the intestine, if the parasite lives freely within it; (2) the type of tissue through which contact is made, i.e., intravascular or interstitial; (3) the length of time this contact is maintained; (4) the size of the parasite and (5) the extent of its muscular development and activity. Given these factors, particularly of close and more prolonged contact of the parasite with the tissues of the host, greater resistance of the latter on the normal diet is due to the sharp cellular reaction about the worm which serves to immobilize, fix and destroy it. In intravascular migrations or habitat, thrombosis and possibly spasm of the vessel are additional factors serving the same purpose. The same applies to muscular ducts, i.e., the nature of their contents as, for example thick viscid mucus, and spasm of the duct itself. The size of the parasite and its muscular power may determine whether it can escape from these restricting

reactive forces. On the deficient diet on the other hand, the reaction on the part of the host is relatively slight and inadequate to immobilize and kill the parasite. Other factors such as atrophy of tissue and possibly disturbances in vaso-or ductal spasm may be contributory factors in permitting more parasites to survive. In our experiments with a deficient diet, we have found that bacterial infection of the parasite itself accounted for considerable mortality. This might superficially give one the impression of increased resistance on the part of the host, which it scarce is, in this instance.

In the available literature dealing with the effects of vitamin A deficiency on helminthic infections Hiraishi,[9] Nagoya,[10] Ackert et. al.,[11] Clapham,[12] McCoy,[13] Foster and Cort,[14] Ogura,[15] Wright,[16] and Spindler[17] demonstrated diminished resistance to infection as compared with controls on adequate diets. These results accord with our findings. But these authors offer little explanation as to why more parasites survive in animals on an inadequate diet. It seems to us that the reasons given above adequately explain this.

Thus, for example, Ackert et. al. reported the development of larger numbers of *Ascaridia lineata* in young chicks on a vitamin A deficient diet and a significantly greater average total worm length per chicken than in the controls. They ascribed the larger number of worms in the animals on the deficient diet to weakened peristalsis. But according to Ackert, *Ascaridia lineata* in chickens has a tissue phase in the host inasmuch as it buries itself into the walls of the duodenum in its early stage of development. It seems to us that this is of greater importance in accounting for the difference in numbers rather than the factor of peristalsis. On the other hand, Clapham found that the vitamin A content of the diet did not directly affect the resistance of chickens against *Heterakis gallinae*. But the latter develops directly in the lumen of the intestine without any tissue phase. In *Parascaris equorum* which does migrate through the tissues of the host before completing its development, she found that the less vitamin A administered in the diet the greater was the survival rate and the more rapid the development of the worms. While Shaw[18] did not find that wethers were more susceptible to lungworm infestations when kept on a diet deficient in vitamin A, his results are open to criticism inasmuch as he used only two animals.

It is not to be implied that the vitamin A factor necessarily in itself accounts for these differences. Indeed, there are reports that dietary deficiencies of other kinds may produce a decreased resistance

of the host. Thus, amongst others Porter[19] found that rats on a whole milk diet developed a reduced resistance to infestation with *Nippostrongylus muris* whether in a primary infection or in the acquired resistance of a reinfection. Foster[20] observed that there was an inverse correlation between anemia and resistance to hookworm infections in dogs and cats. Resistance to *Ancylostomum caninum* could be overcome by periodic bleeding or iron deficiency brought about by a milk diet.

It should finally be added that in one instance a rat depleted of vitamin A was given a single drop of cod liver oil on each of the 1st, 24th and 27th days after infection. Before the second and third supplements its weight had dropped to 75 gms. It had, however, gained 75 gms. three weeks later. When it was killed on the 49th day, the liver grossly resembled the type described in the vitamin A deficient animals. Microscopically, however, there were mixed features of both types, viz., atrophy of the liver cells and even of the small regenerated lobules within and about scars; accentuated thickened portal spaces with apparent proliferation of bile ducts; and moderate lymphocytic infiltration as well as bacteria within biliary radicles, even as in most of the vitamin A deficient animals. On the other hand, there were many recent and organized destructive parasitic lesions with considerable eosinophilic infiltration, and quite marked and diffuse scarring, as in the animals on the normal diet.

This therefore illustrates (1) that sufficient cod liver oil which allows for a good sharp gain in weight augments the degree of parasitic destruction producing lesions of the type seen in the animals on the normal diet; (2) that it is, however, inadequate to stimulate excessive proliferation of liver tissue to the extent of that seen in the controls; and (3) that it is essentially in great part the active regeneration and excessive proliferation of liver tissue in the latter which accentuates the lesions of parasitic destruction rendering the gross changes so distinctive.

SUMMARY

1. White rats were infected with cercariae of *Schistosoma mansoni* and maintained either on a normal diet or a vitamin A free diet, with or without the supplement of one drop of cod liver oil. No attempt was made to determine the effects of the two diets on the parasite during the period of its migration from the skin to the liver.

2. In rats on the normal diet there was marked destruction of the parasites in the liver, maximum between the 5th and 7th weeks

after infection. Thereafter parasites continued to be destroyed in liver and lung until there were few remaining viable worms at the end of the experimental period.

3. In rats on the vitamin A free diet such destruction of the parasites was either absent or minimal. Even with the supplement of a drop of cod liver oil there was relatively little parasitic destruction up to the end of the 6th week after infection. Thereafter greater numbers of dead worms occurred, but still appreciably less than in rats on the normal diet for the same length of time after infection.

4. The reasons for this difference in parasitic survival or so called resistance to the helminthic infection in the animals on the two types of diet are given and are applied to the problems of resistance in relation to diet in helminthic infections in general.

5. While the deficiency of the vitamin A factor in the diet permits excessive proliferation of less highly organized cells such as fibroblasts and histiocytes, it retards the resolution of lesions formed chiefly by these elements (although the foreign body such as the dead parasite may be rapidly digested). It also retards the proliferation of more highly organized cells such as those of the parenchyma of the liver, over and above the minimal requirements of immediate regeneration.

6. In schistosomal infections of the rat it is estimated that a maximum of 24 per cent and an average of 11 per cent of the cercariae to which the animal is exposed will reach the liver. Loss of cercariae through failure to penetrate the skin and beyond that in the complicated pathways until it reaches the liver accounts for this relatively low percentage.

7. A modification of the slide method for the examination of *Trichinella Spiralis* was employed, which is adaptable for determining live and dead worms in the tissues of the infected host. It should be applicable to related studies in other helminthic infections.

We are greatly indebted to Mr. J. L. Janer for his technical assistance.

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APPENDIX

TABLE 1

*6 Rats, 3 on Normal and 3 on a Vitamin A Free Diet Exposed to 3900–4000 Cercariae**

No.	*Sex*	*Diet*	*Age at time of infection in days*	*Number of days (after infection) animal was killed*	*Length of time of depletion on vitamin A deficient diet at time of death in days*	*Supplement*	*Worm distribution*					*Estimated total number live worms*	*Estimated total number dead worms*	*Estimated total of all worms*	*% of total number of worms to number of cercariae exposed*
							Heart	*Lung*	*Mesentery*	*Liver*					
										Alive	*Dead*				
66	F	A free	83	28	83	0	0	1	2	330	1	333	1	334	8.45
67	M	Normal	42	29	—	—	2	0	12	382	171	396	171	567	14.35
153	M	Normal	42	85	—	—	0	9	0	16	45	16	54	70	1.77
162	M	Normal	42	108	—	—	0	10	3	21	13	24	23	47	1.19

* 2 Rats on the vitamin A free diet died 17 and 21 days after infection respectively. Estimated number of cercariae 3900—check count 3916.

TABLE 2

*10 Rats, 5 on Normal and 5 on a Vitamin A Free Diet Exposed to 3700–3800 Cercariae**

No.	*Sex*	*Diet*	*Age at time of infection in days*	*Number of days (after infection) animal was killed*	*Length of time of depletion on vitamin A deficient diet at time of death in days*	*Supplement*	*Worm distribution*					*Estimated total number live worms*	*Estimated total number dead worms*	*Estimated total of all worms*	*% of total number of worms to number of cercariae exposed*
							Heart	*Lung*	*Mesentery*	*Liver*					
										Alive	*Dead*				
69	M	A free	131	29	132	0	0	0	25	541	2	566	2	568	15.14
70	M	A free	84	30	86	0	0	0	3	235	0	238	0	238	6.34
93	M	A free	106	42	120	0	0	0	0	381	0	381	0	381	10.15
72	F	Normal	43	32	—	—	0	2	2	199	366	201	368	569	15.17
86	M	Normal	43	39	—	—	2	1	4	178	387	184	388	572	15.25
94	F	Normal	42	42	—	—	0	1	0	104	208	105	208	313	8.34
149	M	Normal	42	79	—	—	0	22	5	150	192	155	214	369	9.84
163	M	Normal	43	108	—	—	0	34	4	83	25	87	59	146	3.89

* 2 rats on vitamin A free diet died 3 and 29 days after infection respectively. Estimated number of cercariae 3630—check count 3760.

TABLE 3

*15 Rats, 7 on Normal and 8 on a Vitamin A Free Diet Exposed to 3900–4100 Cercariae**

No.	Sex	Diet	Age at time of infection in days	Number of days (after infection) animal was killed	Length of time of depletion on vitamin A deficient diet at time of death in days	Supplement	Worm distribution					Estimated total number live worms	Estimated total number dead worms	Estimated total of all worms	% of total number of worms to number of cercariae exposed
							Heart	Lung	Mesentery	Liver					
										Alive	Dead				
†71	F	A free	107	29	108	0	0	0	13	197	10	210	10	220	5.5
73	M	A free	108	30	110	0	0	0	0	531	4	531	4	535	13.37
80	M	A free	107	34	113	0	0	0	5	372	0	377	0	377	9.42
81	F	A free	105	34	111	0	0	0	0	210	24	210	24	234	5.85
82	F	A free	108	35	115	0	0	0	8	356	0	364	0	364	9.10
95	F	A free	105	41	118	0	0	0	0	245	1	245	1	246	6.15
98	F	A free	108	42	122	0	0	0	0	267	0	267	0	267	6.67
78	F	Normal	45	33	—	—	0	2	3	245	177	251	177	428	10.70
84	F	Normal	44	36	—	—	0	0	0	186	189	186	189	375	9.37
91	M	Normal	44	39	—	—	0	2	0	111	221	113	221	334	8.35
96	M	Normal	44	41	—	—	0	0	0	99	92	99	92	191	4.77
104	F	Normal	45	47	—	—	1	0	4	120	298	125	298	423	10.57
148	M	Normal	44	76	—	—	0	10	0	55	73	55	83	138	3.45
164	F	Normal	45	107	—	—	0	3	0	28	29	28	32	60	1.50

* One rat on the vitamin A free diet died 35 days after infection but was not examined for number and distribution of schistosomes. Estimated number of cercariae 3900–4100—check count 4092.

† Found dead in well preserved fresh state.

TABLE 4

*14 Rats, 6 on Normal and 8 on a Vitamin A Free Diet Exposed to 3900–4100 Cercariae**

No.	Sex	Diet	Age at time of infection in days	Number of days (after infection) animal was killed	Length of time of depletion on vitamin A deficient diet at time of death in days	Supplement	Worm distribution					Estimated total number live worms	Estimated total number dead worms	Estimated total of all worms	% of total number of worms to number of cercariae exposed
							Heart	Lung	Mesentery	Liver					
										Alive	Dead				
†83	M	A free	106	15	93	0	0	0	0	218	0	218	0	218	5.45
99	M	A free	125	23	120	0	0	0	5	523	0	528	0	528	13.20
121	M	A free	75	36	83	0	1	2	15	605	1	623	1	624	15.60
101	M	Normal	65	24	—	—	0	0	5	443	184	448	184	632	15.80
123	M	Normal	65	37	—	—	0	1	1	208	342	210	342	552	13.80
137	M	Normal	65	43	—	—	0	1	0	134	244	134	245	379	9.47
154	F	Normal	65	63	—	—	3	17	5	89	75	97	92	189	4.72
161	M	Normal	65	84	—	—	0	57	0	122	114	122	171	293	7.32
165	M	Normal	64	111	—	—	1	14	1	47	56	49	70	119	2.97

* 5 rats on vitamin A free diet died 10, 13, 14, 19 and 29 days after infection, respectively, but were not examined for number and distribution of worms. Estimated number of cercariae 3875—check count 4042.

† In moribund condition when killed.

TABLE 5

*12 Rats, 6 on Normal and 6 on a Vitamin A Free Diet Exposed to 3500–3600 Cercariae**

No.	Sex	Diet	Age at time of infection in days	Number of days (after infection) animal was killed	Length of time of depletion on vitamin A deficient diet at time of death in days	Supplement	Worm distribution					Estimated total number live worms	Estimated total number dead worms	Estimated total of all worms	% of total number of worms to number of cercariae exposed
							Heart	Lung	Mesentery	Liver					
										Alive	Dead				
107	M	A free	77	27	76	1 drop c.l.o.† 2 days b.k.‡	0	0	0	244	6	244	6	250	7.04
109	M	A free	77	28	77	1 drop c.l.o. 3 days b.k.	0	0	13	446	35	459	35	494	13.91
138	M	A free	77	42	91	1 drop c.l.o. 17 days b.k.	0	1	1	322	5	323	6	329	9.26
151	F	A free	77	57	106	1 drop c.l.o. 32 days b.k.	8	18	85	570	29	672	38	710	20.00
157	M	A free	77	69	118	1 drop c.l.o. 44 days b.k.	40	146	0	367	70	480	142	622	17.52
110	M	Normal	67	28	—	—	0	0	3	268	83	271	83	354	9.97
134	F	Normal	66	40	—	—	0	0	3	201	335	204	335	539	15.18
140	F	Normal	67	43	—	—	0	0	0	112	311	112	311	423	11.91
150	F	Normal	66	56	—	—	0	6	2	77	153	79	159	238	6.70
158	F	Normal	67 (?)	70	—	—	0	20	0	32	80	42	90	132	3.71
166	M	Normal	(?)	115	—	—	0	0	0	16	32	16	32	48	1.35

* One rat on vitamin A free diet died 24 days after infection and no worm counts were made. Estimated number of cercariae 3570—check count 3569.
† c.l.o. = cod liver oil.
‡ b.k. = before killed.

TABLE 6

*16 Rats, 8 on Normal and 8 on a Vitamin A Free Diet Exposed to 3800–4000 Cercariae**

No.	*Sex*	*Diet*	*Age at time of infection in days*	*Number of days (after infection) animal was killed*	*Length of time of depletion on vitamin A deficient diet at time of death in days*	*Supplement*	*Worm distribution*					*Estimated total number live worms*	*Estimated total number dead worms*	*Estimated total of all worms*	*% of total number of worms to number of cercariae exposed*
							Heart	*Lung*	*Mesentery*	*Liver*					
										Alive	*Dead*				
103	M	A free	102	20	94	0	0	0	0	380	0	380	0	380	9.74
111	M	A free	88	25	85	1 drop c.l.o.† 1 day b.k.‡	0	0	2	267	1	269	1	270	6.92
117	F	A free	102	28	102	1 drop c.l.o. 4 days b.k.	0	0	0	694	8	694	8	702	18.00
118	F	A free	88	28	88	1 drop c.l.o. 4 days b.k.	0	0	1	434	0	435	0	435	11.15
125	F	A free	88	32	92	1 drop c.l.o. 8 days b.k.	0	0	0	801	0	801	0	801	20.53
128	F	A free	102	34	108	1 drop c.l.o. 10 days b.k.	0	0	0	936	0	936	0	936	24.00
145	F	A free	102	42	116	1 drop c.l o. 18 days b.k.	0	0	8	750	66	758	66	824	21.12
155	F	A free	88	63	123	1 drop c.l.o. 39 days b.k.	16	124	2	273	100	295	220	515	13.20

TABLE 6 (*Continued*)

No.	*Sex*	*Diet*	*Age at time of infection in days*	*Number of days (after infection) animal was killed*	*Length of time of depletion on vitamin A deficient diet at time of death in days*	*Supplement*	*Worm distribution*					*Estimated total number live worms*	*Estimated total number dead worms*	*Estimated total of all worms*	*% of total number of worms to number of cercariae exposed*
							Heart	*Lung*	*Mesentery*	*Liver*					
										Alive	*Dead*				
102	M	Normal	71	19	—	—	0	0	0	351	56	351	56	407	10.43
114	F	Normal	71	26	—	—	0	0	8	120	17	128	17	145	3.71
119	M	Normal	71	29	—	—	0	0	1	396	4	397	4	401	10.28
127	F	Normal	71	33	—	—	0	0	3	332	130	335	130	465	11.92
144	M	Normal	71	41	—	—	0	3	5	231	227	238	228	466	11.95
**147	F	Normal	71	49	—	—	0	2	3	89	84	92	86	178	4.56
**156	F	Normal	71	64	—	—	7	30	0	135	126	142	156	298	7.64
167	F	Normal	71	112	—	—	0	15	1	35	36	36	51	87	2.23

*Estimated number of cercariae 3800–4000. Check count 3792.

** Found to be pregnant after infection. Both gave birth to litters of 2 and 7 young, respectively. These young were killed but no schistosomes were encountered in their livers.

† c.l.o. = cod liver oil.

‡ b.k. = before killed.

DESCRIPTION OF PHOTOMICROGRAPHS

FIGURES 1 to 8 (incl.) represent the various ways in which the parasite is fixed within peripheral portal veins prior to its destruction in the rat on the normal diet.

FIGURES 9 to 15 (incl.) represent the various ways in which the parasite once killed is disposed of and some of the effects on the surrounding liver substance in the rat on the normal diet.

FIGURES 16 to 24 (incl.) include the modes of destruction of the parasite and the types of reaction evoked by their presence, destruction or oviposition in the later stages of infection in the rat on the vitamin A deficient diet. Earlier stages are omitted as they almost uniformly present viable worms in peripheral veins with little surrounding reaction.

MICROFOTOGRAFÍAS

GRABADO 1-8 (inclusive). Distintas formas en que el parásito queda fijado dentro de las venas portales periféricas antes de ser destruído, en un animal con alimentación normal.

GRABADO 9-15 (incl.). Distintas formas en que el parásito muerto desaparece y efectos que se observan en el parenquima hepático circundante, en un animal con alimentación normal.

GRABADO 16-24 (incl.). Distintas formas en que el parásito queda destruído y tipos de reacción celular que provoca su presencia, su destrucción, o la presencia del huevo, en las últimas etapas de la esquistosomización de un animal con alimentación deficiente en vitamina A. (En las primeras etapas casi no se observan más que vermes vivos en las venas periféricas, con muy poca reacción circundante.)

FIGURE 1: The parasite is wedged within the portal vein by a thrombus of eosinophilic cells at the junction of the vein with a tributary. Note the thickened portal space heavily infiltrated by eosinophils. (Normal diet, 35 days after infection) x 450.

GRABADO 1: El parásito está atrapado dentro de la vena porta por un trombo de eosinófilos, en la bifurcación de una vena secundaria. Nótese el espesor del espacio portal, intensamente infiltrado de eosinófilos. (Animal a dieta normal, 35 días después de esquistosomizado) x 450.

FIGURE 2: The parasite is wedged between the wall of the vein on the right and a projecting shelf of eosinophilic infiltration to the left, over which there is little recognizable endothelium. The narrow clear spaces between worm and venous walls are artefacts due to fixation. There are red cells within and patency of the lumen only above and to the left. (Normal diet, 35 days after infection) x 450.

GRABADO 2: El parásito está atrapado entre la pared de la vena, a la derecha, y una capa de infiltración eosinófila, a la izquierda, distinguiéndose apenas el endotelio. Los espacios claros y estrechos entre el verme y las paredes venosas son producidos por el aparato de fijación. Existen corpúsculos rojos dentro del lumen del vaso que va por encima y a la derecha. (Animal con alimentación normal, 35 días después de esquistosomizado) x 450.

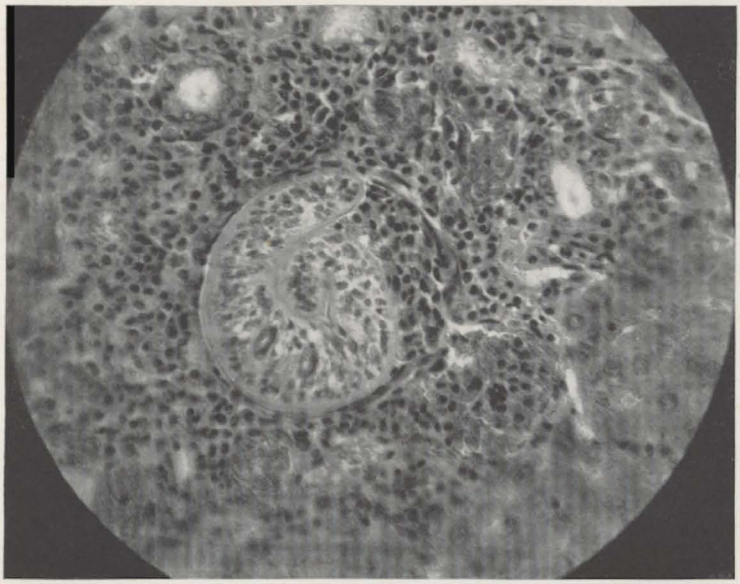

FIGURE 1

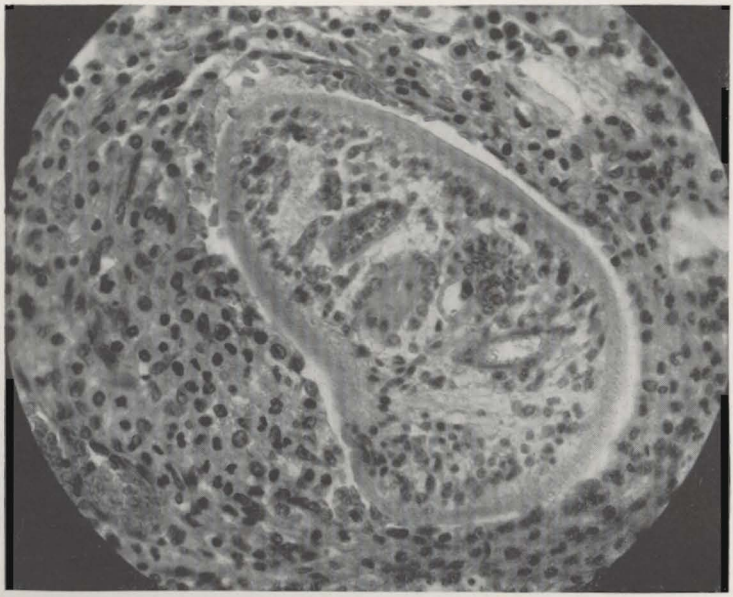

FIGURE 2

FIGURE 3: The parasite is squeezed and misshapen within a portal vein. There is a thrombus of eosinophils completely occluding the lumen of the vein between the limbs of the Y-form of the parasite above and to the left. Below and to the right there is loss of endothelium and the eosinophils of the parietes lie against the cuticle of the worm. There are two areas of parenchymatous necrosis, the larger one to the right of the portal space, the smaller one to the left reaches the portal space. (Normal diet, 35 days after infection) x 100.

GRABADO 3: Parásito comprimido y retorcido dentro de una vena portal. Un trombo de eosinófilos obstruye completamente el lumen de la vena entre las dos ramas de Y que forma el parásito por encima y a la izquierda. Por debajo y a la derecha hay pérdida del endotelio, y los eosinófilos yacen contra la cutícula del verme. Hay dos áreas de necrosis parenquimatosa, la mayor a la derecha del espacio portal; la menor, a la izquierda, llega hasta el espacio portal. (Animal con alimentación normal, 35 días después de esquistosomizado) x 100.

FIGURE 4: Note how the paired parasites are being enclosed within the vascular lumen by a fibrinous thrombus to the left. The thrombus arises from the mouth of a collateral vein and shows evidences of early organization. Note its smooth surface where the parasite probably moved against it and its rough opposite surface. The thrombus is not covered by endothelium. (Normal diet, 42 days after infection) x 100.

GRABADO 4: Nótese cómo los parásitos apareados quedan presos dentro del lumen vascular por un trombo fibrinoso a la izquierda, que sale de la boca de una vena colateral, y presenta signos de organización reciente. Véase la superficie suave, contra la cual probablemente se debatió el parásito, y la aspereza de la superficie opuesta. El trombo no está cubierto por endotelio. (Animal con alimentación normal, a los 42 días de esquistosomizado) x 100.

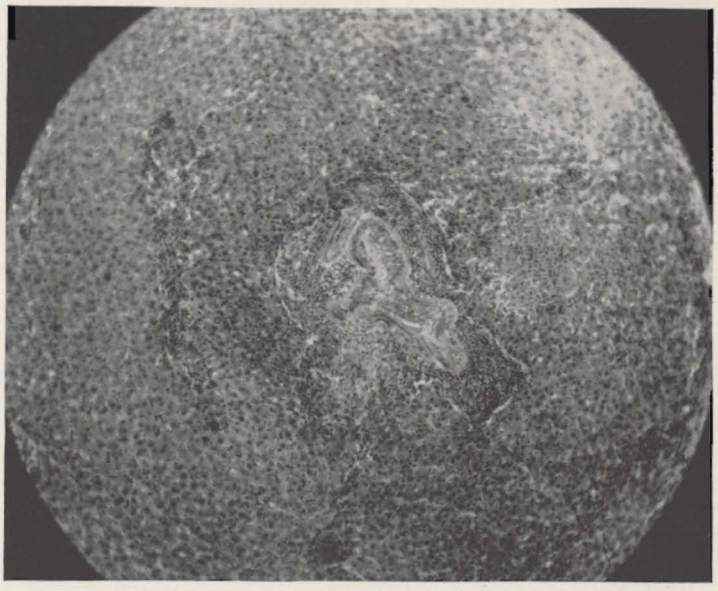

FIGURE 3

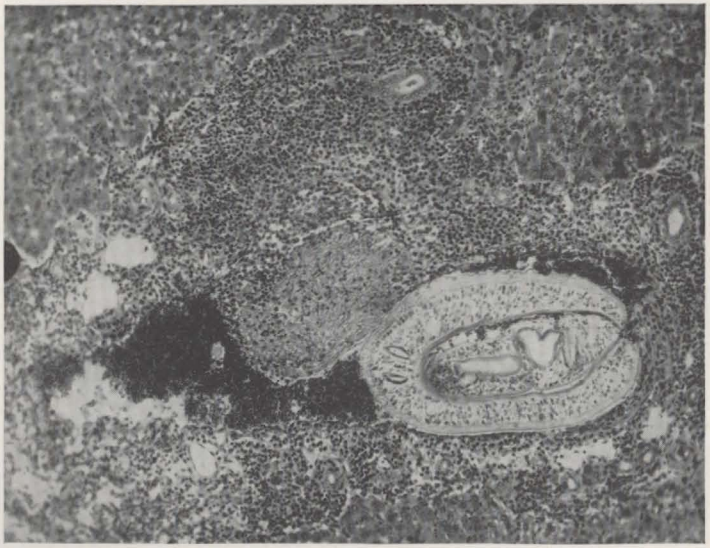

FIGURE 4

FIGURE 5: Note how tightly wedged the viable parasite is between the organizing destroyed parasitic lesion above and the infiltrated venous wall below. The clear spaces are artefacts due to fixation and shrinkage. (Normal diet, 42 days after infection) x 100.

GRABADO 5: Véase la compresión de un parásito vivo entre una lesión de un parásito destruído arriba y la infiltración de la pared venosa debajo. Los espacios claros se deben a los elementos de fijación y a la constricción que sufre el tejido. (Animal con alimentación normal, 42 días después de esquistosomizado) x 100.

FIGURE 6: A viable paired couple lying in a wide subcapsular vein are seen. Note the fixation of the male to the wall of the vein to the left, near a parietal deposit of ova surrounded by fibroblasts. (Normal diet, 63 days after infection) x 100.

GRABADO 6: Dos parásites vivos apareados en una espaciosa vena subcapsular. Nótese cómo el macho se adhiere a la pared en la vena que queda a la derecha, cerca de un depósito de huevecillos junto a la pared, rodeado por fibroblastos. (Animal con alimentación normal, 63 días después de esquistosomizado) x 100.

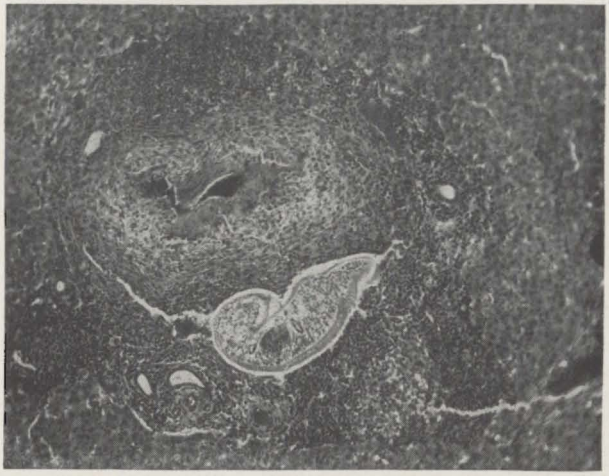

FIGURE 5

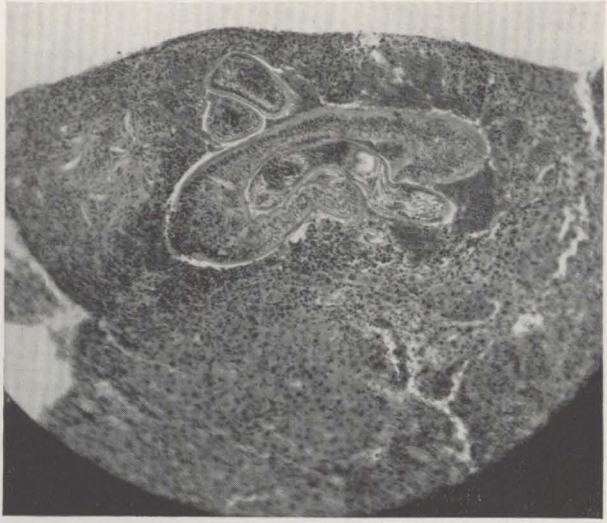

FIGURE 6

FIGURE 7: Same as Figure 6, but high power area of fixation of the male parasite is represented. Note the loss of cuticle and invasion of the substance of the parasite by cells of the host. The point of anchorage to the vessel wall has been ruptured probably agonally and there is a small hemorrhage at its site x 1000.

GRABADO 7: El mismo grabado anterior, a gran aumento: área de fijación del macho. Nótese la pérdida de la cutícula y la invasión celular del parásito. El punto en que el parásito se adhiere a la pared vascular se ha roto, probablemente con los movimientos agónicos, y ha producido una pequeña hemorragia.

FIGURE 8: There is a massive thrombus of red blood cells and eosinophils crowding the parasite against the inferior part of the vessel. There is no invasion of the worm by cells of the host. The frayed appearance of the parasite in that sector of it above its main portion, completes its smooth contour in a slightly higher plane of focus in the section from which this was taken. (Normal diet, 35 days after infection) x 450.

GRABADO 8: Gran trombo de hematíes y eosinófilos que empujan al parásito contra la porción inferior del vaso. No hay invasión celular del verme. El aspecto deshilachado, en el segmento por encima de la porción principal del parásito, se torna de contorno suave en un plano superior al enfocado en este corte histológico. (Animal con alimentación normal, a los 35 días después de esquistosomizado) x 450.

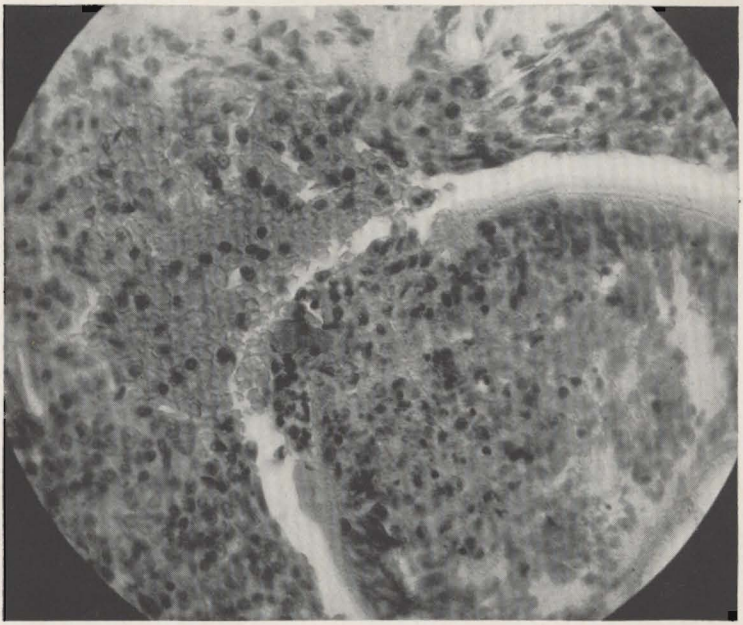

FIGURE 7

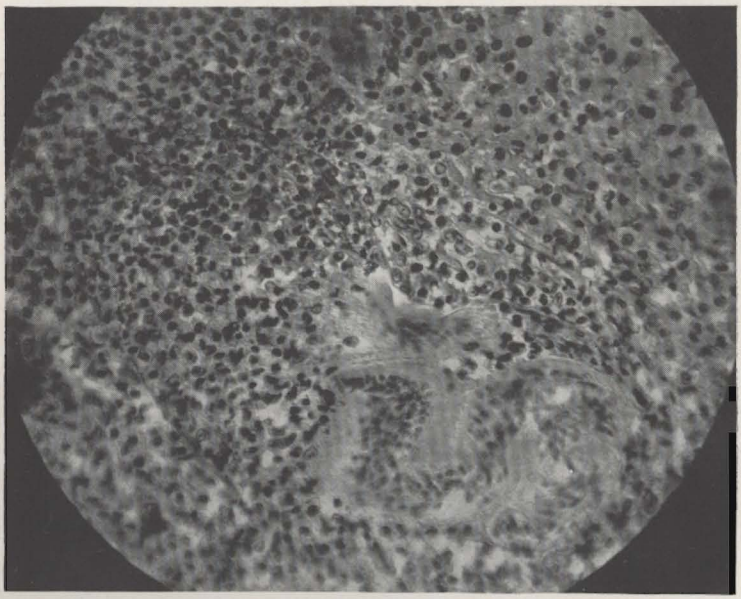

FIGURE 8

FIGURE 9: The recently dead parasite is surrounded by eosinophils which in turn are enclosed by an extensive fibrinous thrombus with a fibroblastic border below and to the right. (Normal diet, 35 days after infection) x 100.

GRABADO 9: Un parásito acabado de fenecer, rodeado por eosinófilos, que a su vez están englobados en un gran trombo fibrinoso, con un borde fibroblástico por debajo y a la derecha. (Animal con alimentación normal, a los 35 días de esquistosomizado) x 100.

FIGURE 10: There is massive eosinophilic infiltration of the necrotic worm and within the eosinophilic thrombus about it there is still a well preserved sector of worm above and to the left. (Normal diet, 35 days after infection) x 450.

GRABADO 10: Infiltración masiva eosinófila de un verme necrótico. Dentro del trombo eosinófilo, hacia arriba y a la derecha, hay aún un pedazo, bien conservado, del verme.

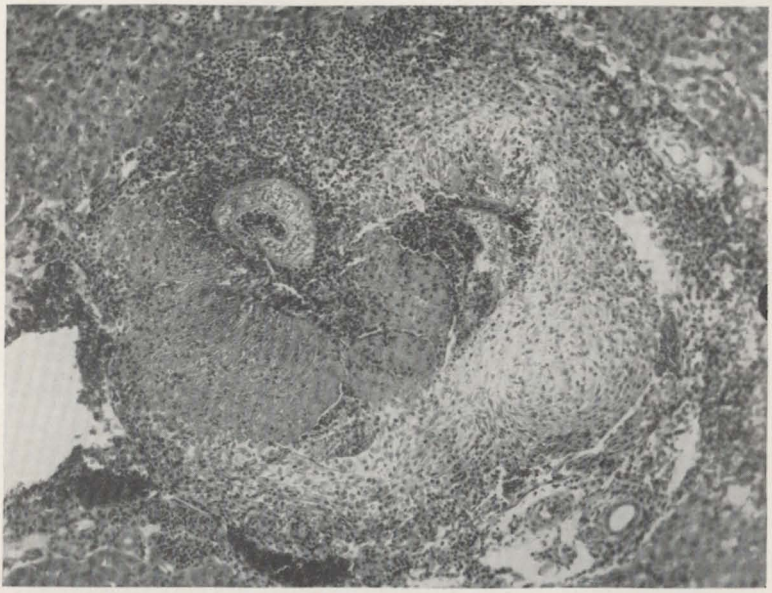

FIGURE 9

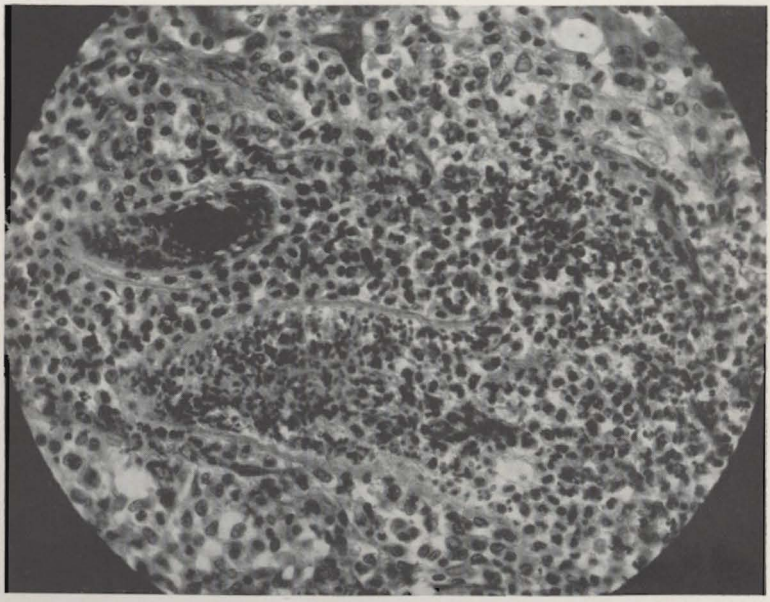

FIGURE 10

FIGURE 11: To demonstrate the extensive zone of necrosis of liver about a portal space in which a worm has been destroyed. (Normal diet, 35 days after infection) x 100.

GRABADO 11: Extensa zona necrótica en el hígado, próxima al espacio portal, en la cual ha quedado destruído un verme. (Animal con alimentación normal, a los 35 días después de esquistosomizado) x 100.

FIGURE 12: There is a ring of fibroblasts about a dead worm with masses of eosinophils separating worm and fibrous wall. Note the enlargement of the surrounding collateral plexus of veins. (Normal diet, 35 days after infection) x 100.

GRABADO 12: Anillo de fibroblastos en torno a un verme muerto, con grupos de eosinófilos separando el verme de la pared fibrosa. Nótese engrosamiento del plexo venoso colateral en torno. (Animal con dieta normal, a los 35 días después de esquistosomizado) x 100.

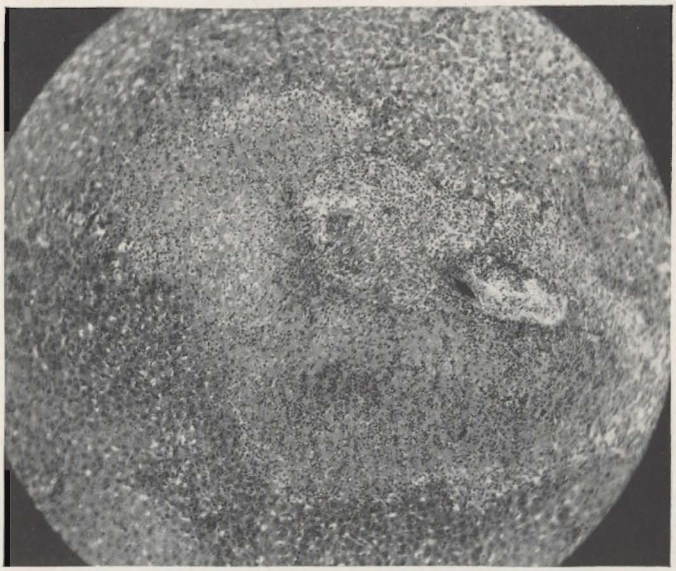

FIGURE 11

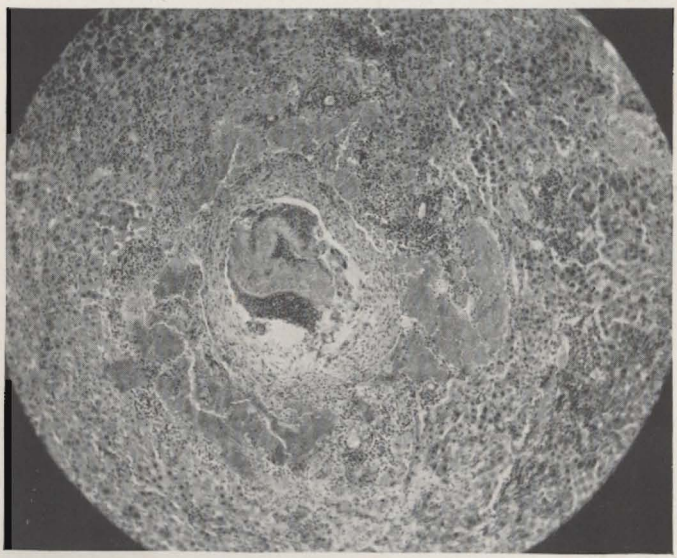

FIGURE 12

FIGURE 13: There is a subcapsular fibrous nodule of an organized destroyed parasite with subcapsular proliferation of liver cells. Note the raised cobbled effect. (Normal diet, 35 days after infection) x 100.

GRABADO 13: Nódulo fibroso subcapsular formado en el lugar que ocupaba un parásito destruído, con proliferación subcapsular de las células hepáticas. Nótese el aspecto pizarroso y ondulado. (Animal con alimentación normal, a los 35 días después de esquistosomizado) x 100.

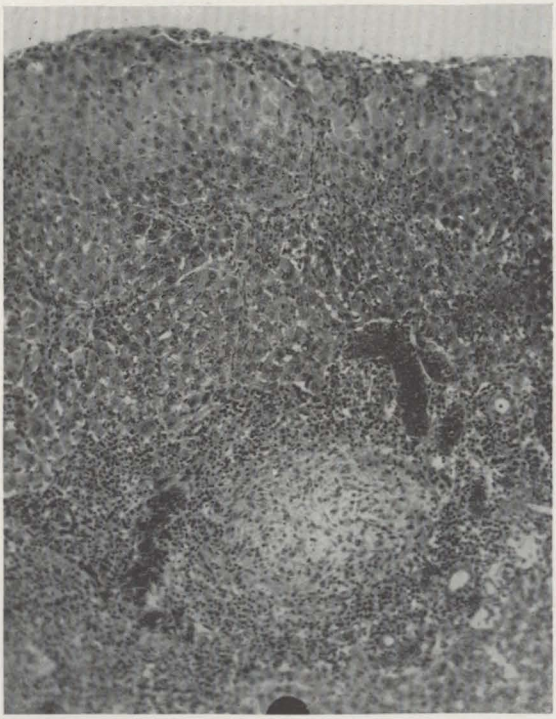

FIGURE 13

FIGURE 14: Slow fibrous and monocytic organization of a hyalinized necrotic parasite in the late stages of infection. (Normal diet, 113 days after infection) x 100.

GRABADO 14: Proceso lento de organización fibrosa y monocitos de un parásito hialinizado, en las últimas etapas de la esquistosomización. (Animal con alimentación normal, a los 113 días después de esquistosomizado) x 100.

FIGURE 15: To demonstrate nests of eggs and how minimal the reaction about them may be. (Normal diet, 63 days after infection) x 100.

GRABADO 15: Nidos de huevecillos. Véase la insignificante reacción celular en torno. (Animal con alimentación normal, a los 63 días de esquistosomizado) x 100.

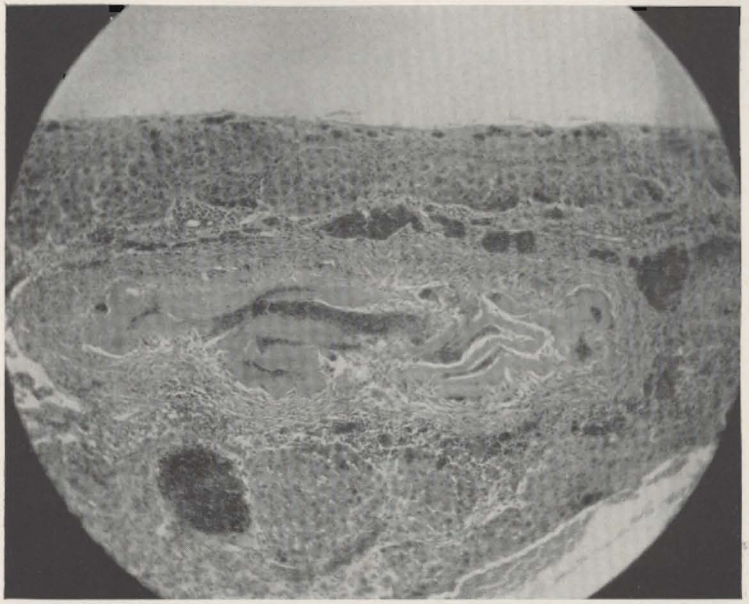

FIGURE 14

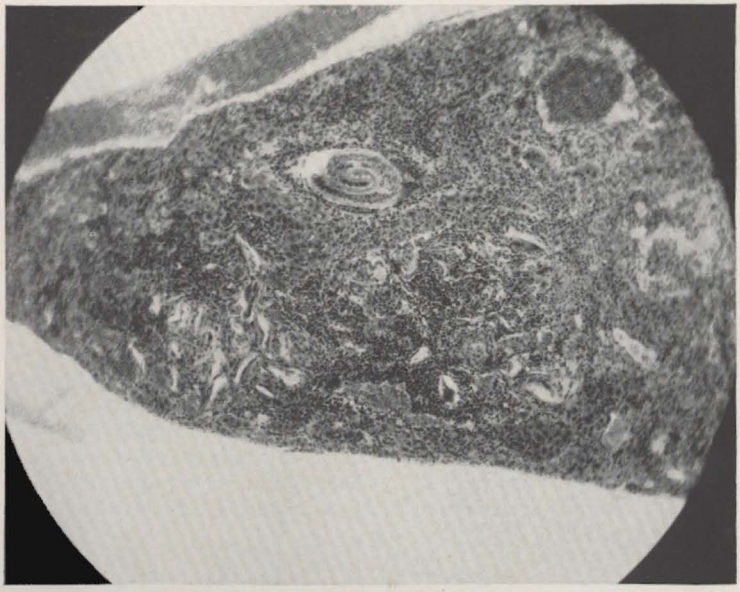

FIGURE 15

FIGURE 16: There is massing of parasites within a peripheral portal vein. Note the relatively small amount of round cell infiltration of the portal space. (42 days after infection. Vitamin A free diet with supplement of 1 drop of cod liver oil 10 days before being killed) x 100.

GRABADO 16: Masas de parásitos dentro de un vaso portal periférico. Nótese la pequeña cantidad de células redondas de infiltración en los espacios portales. (Animal con alimentación privada de vitamina A, a los 42 días de esquistosomizado, con alimentación suplementaria de una gota diaria de aceite de hígado de bacalao, 10 días antes de ser sacrificado) x 100.

FIGURE 17: Note to the left the large fibroblastic nodules of reaction about a destroyed parasite, the only remains of which are the large clumps of pigment. Note how these project into the remaining patent lumen of the vessel. To the right there is a parasite squeezed within a portal space due to: (1) A fibroblastic shelf above and to the left of the three sectors of the worm, (2) a thrombus immediately below the fibroblastic shelf which completes the closure of the lumen. The thrombus is made up of a crescentic loop of fibrin and otherwise heterophile polymorphonuclears. (Same animal as in Fig. 16) x 100.

GRABADO 17: Véanse a la izquierda los grandes nódulos fibroblásticos de reacción, en el lugar que ocupaba el parásito destruído. Los únicos vestigios del verme son los grumos de pigmento, que se proyectan dentro del lumen del vaso. A la derecha hay un parásito comprimido, dentro de un espacio portal, por una capa fibroblástica por encima, a la izquierda de las tres cuartas partes del verme, y por un trombo situado inmediatamente por debajo de la capa fibroblástica que acaba cerrando la luz del vaso. El trombo está constituído por fibrina y polinucleados. (El mismo animal que en el grabado anterior) x 100.

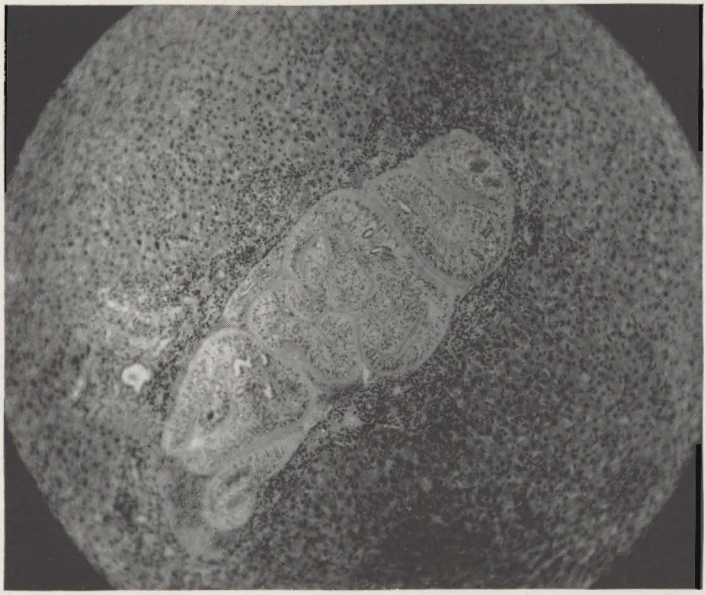

FIGURE 16

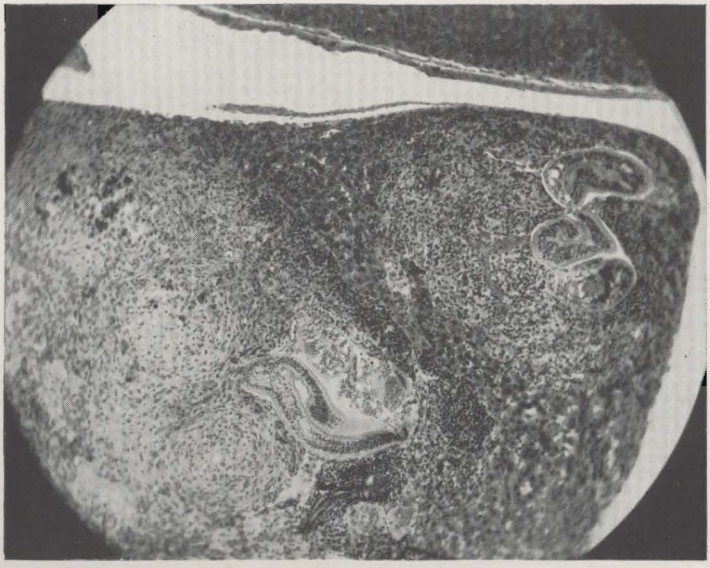

FIGURE 17

FIGURE 18: Note the exaggerated fibroblastic nodules about ova, and viable parasites wedged in the portal space between shelves formed by these nodules and the vascular wall below and to the right. Note the enlarged ceca of the parasites which are filled with bacteria. (65 days after infection Vitamin A free and supplemented with 1 drop of cod liver oil 33 days before being killed) x 100.

GRABADO 18: Nótense los enormes nódulos fibroblásticos en torno a los huevecillos y los parásitos vivos apretados entre estos mismos nódulos y la pared vascular. Véase el ciego hipertrófico de los parásitos, lleno de bacterias. (Animal con alimentación privada de vitamina A, 65 días después de esquistosomizado, con alimentación suplementaria de una gota diaria de aceite de hígado de bacalao, durante 33 días antes de ser sacrificado) x 100.

FIGURE 19: High power of intestinal ceca of parasite filled with bacilli. (Same animal as in Figure 18) Giemsa x 1000.

GRABADO 19: Ciego intestinal del parásito, a gran aumento, lleno de bacilos. (El mismo animal anterior) Col. Giemsa; x 1,000.

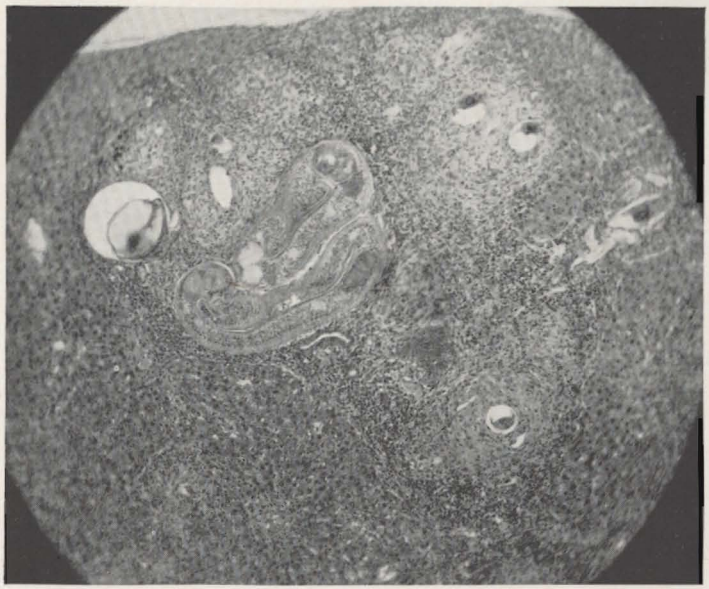

FIGURE 18

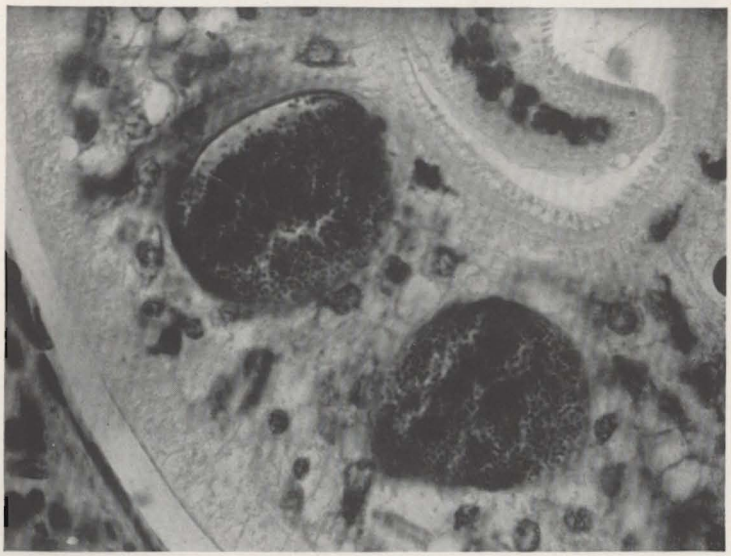

FIGURE 19

FIGURE 20: The parasites are converted into bacterial filled cysts. (Same animal as in Figure 18) x 100.

GRABADO 20: Los parásitos quedan convertidos en quistes repletos de bacterias. (El mismo animal anterior) x 100.

FIGURE 21: Rupture of bacterial filled cecal cysts with resulting pylephlebitic abscess. (Same animal as in Fig. 18) x 100.

GRABADO 21: Ruptura de los quiestes cecales bacterianos, con la fileflebitis resultante. (El mismo animal anterior) x 100.

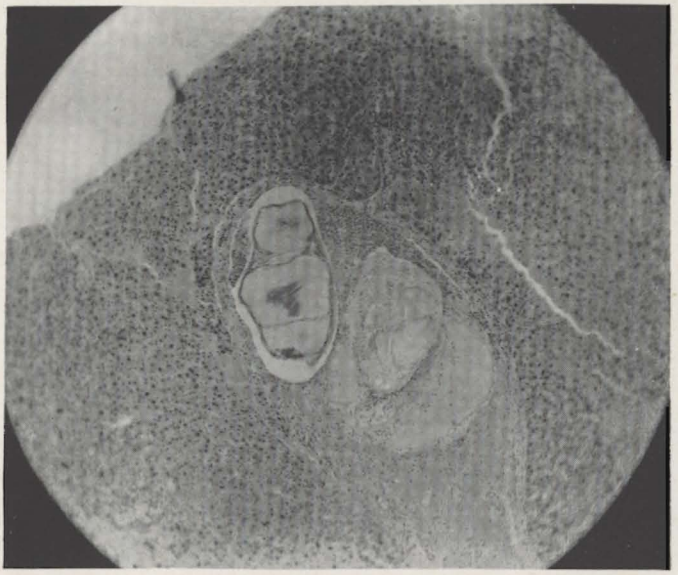

FIGURE 20

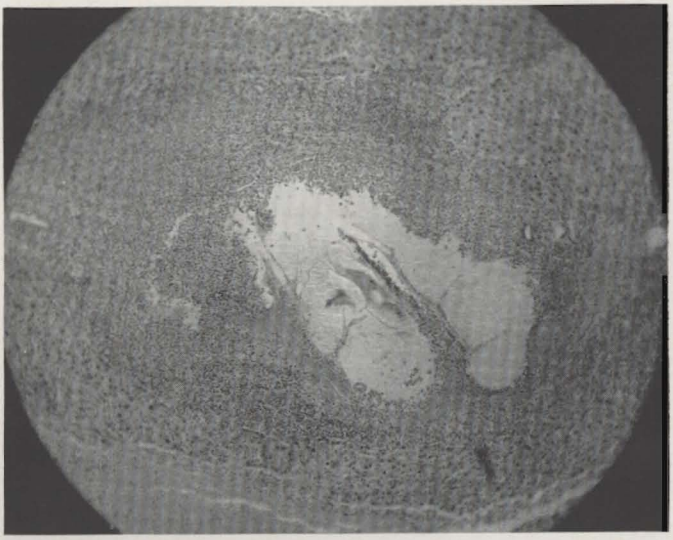

FIGURE 21

FIGURE 22: Parasite in branch of pulmonary artery with early bacterial invasion of ceca. The black clumps within the ceca are remains of pigment. (Same animal as in Figure 18) x 450.

GRABADO 22: Parásito en una rama de la arteria pulmonar, con el ciego invadido prematuramente por bacterias. Los montones negros en el ciego son restos del pigmento. (El mismo animal anterior) x 450.

FIGURE 23: Above center of section of lung there is a large nodule in which there is no trace of parasite but remains of a central abscess and surrounding monocytic, fibroblastic and lymphocytic reaction. (Vitamin A free diet—no supplement; 45 days after infection) x 100.

GRABADO 23: Encima del centro de un corte de tejido pulmonar aparece un nódulo grande, sin trazas del parásito, pero hay un absceso central y una reacción en torno, con monocitos, fibroblastos y linfocitos. (Animal con alimentación sin vitamina A, no suplementada, a los 45 días de esquistosomizado) x 100.

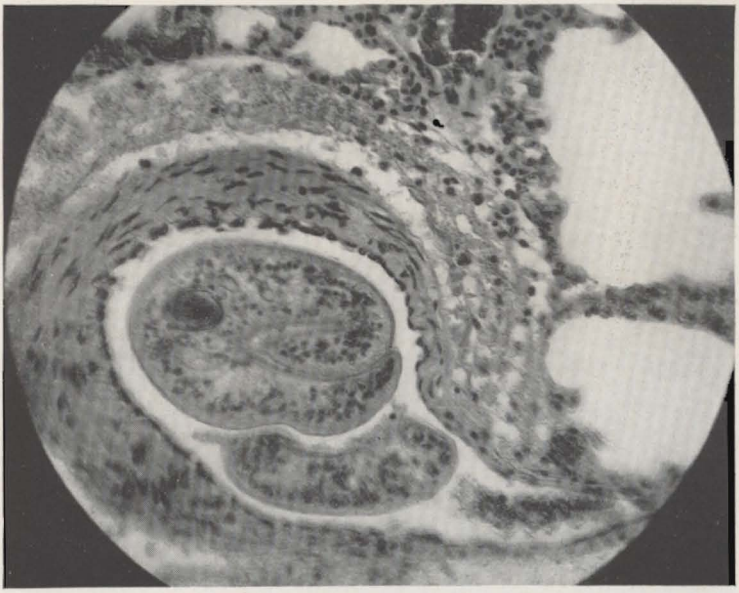

FIGURE 22

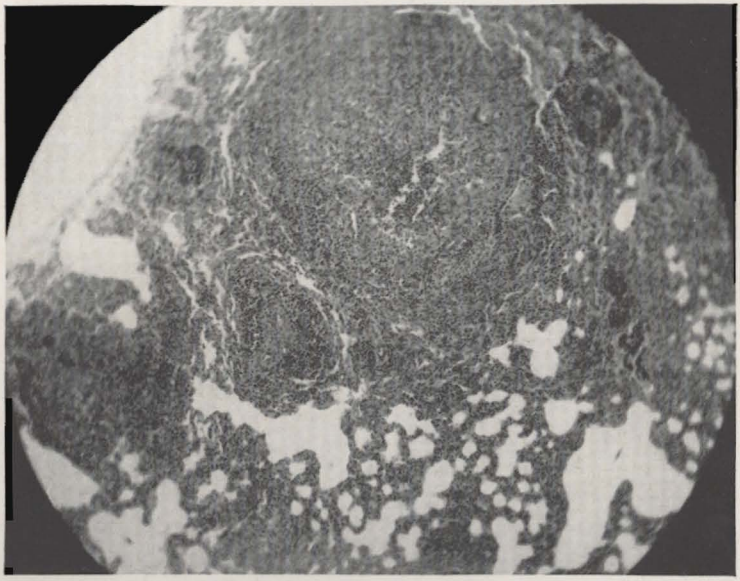

FIGURE 23

FIGURE 24: Necrotic worm which has been ulcerated into the lumen of a bronchus. (Same animal as in Figure 23) x 100.

GRABADO 24: Verme necrótico, con ulceración en el lumen de un bronquio. (El mismo animal anterior) x 100.

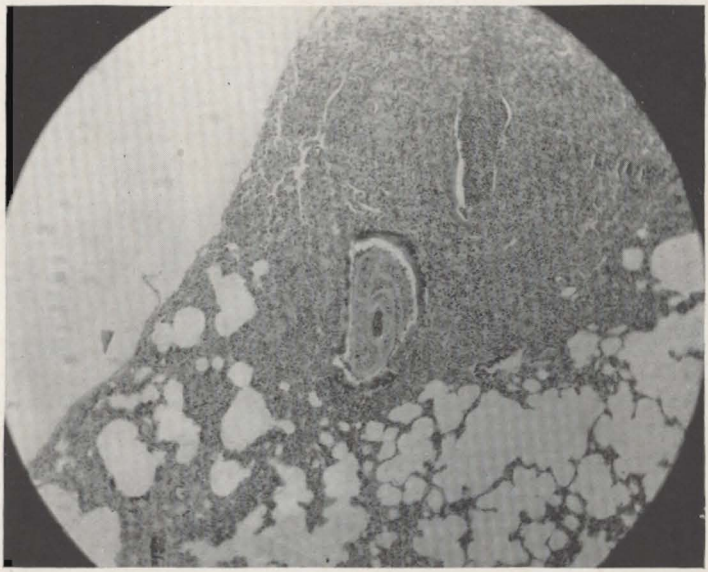

FIGURE 24

FIGURE 25: Liver of infected rat on normal diet, 50 days after infection. Note scarring and lobular hypertrophy or cobbled effect. A starch mixture was used for contrast.

GRABADO 25: Hígado de un animal con alimentación normal, después de 50 días de esquistosomizado. Nótese el aspecto cicatricial y la hipertrofia lobular o aspecto pizarroso. Hemos utilizado una mezcla de almidón para hacer resaltar las alteraciones histológicas.

FIGURE 25

FIGURE 26: Lungs and liver of infected rat on vitamin A deficient diet, 49 days after infection. Note the large multiple pulmonary lesions due to parasitic invasion of the lung and the smooth liver with white marginal and submarginal nodules.

GRABADO 26: Pulmones e hígado de un animal con alimentación deficiente en vitamina A, 49 días después de esquistosomizado. Nótense las lesiones pulmonares múltiples provocadas por la invasión parasitaria del pulmón, y la superficie lisa del hígado con nódulos marginales y submarginales.

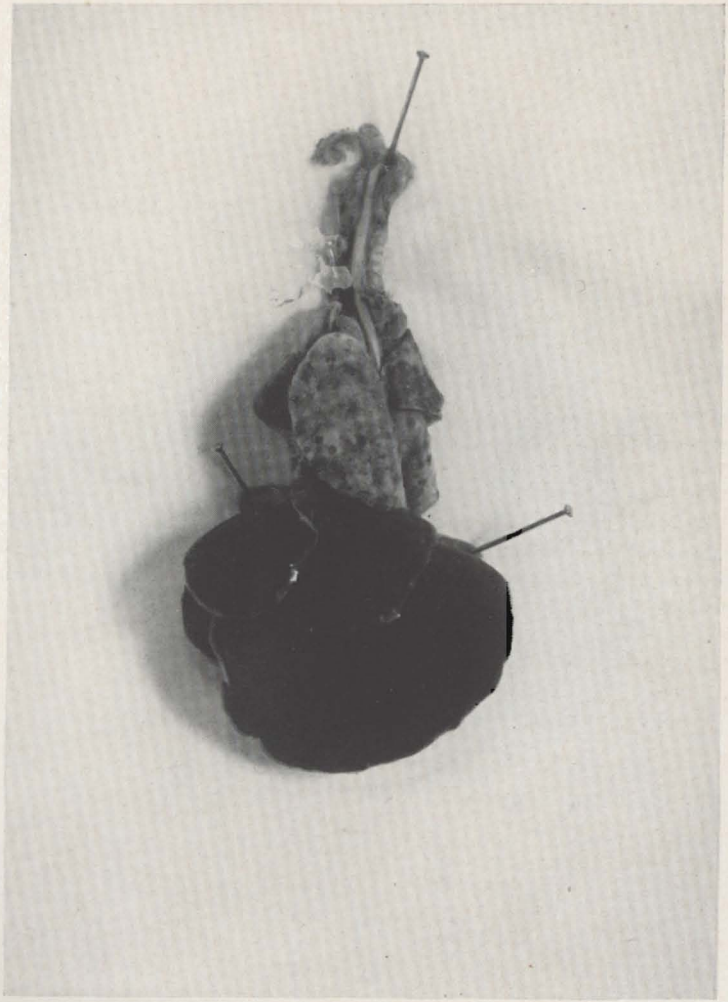

FIGURE 26

FIGURE 27: Liver of infected vitamin A deficient rat, 65 days after infection to show smooth surface and the measled effect (numerous small white spots) of oviposition chiefly but also of parasitic massing and destruction.

GRABADO 27: Hígado de un animal sometido a alimentación deficiente en vitamina A, después de 65 días de esquistosomizado. Nótese la superficie lisa y con profuso moteado blanco debido a la oviposición y a la destrucción parasitaria en masa.

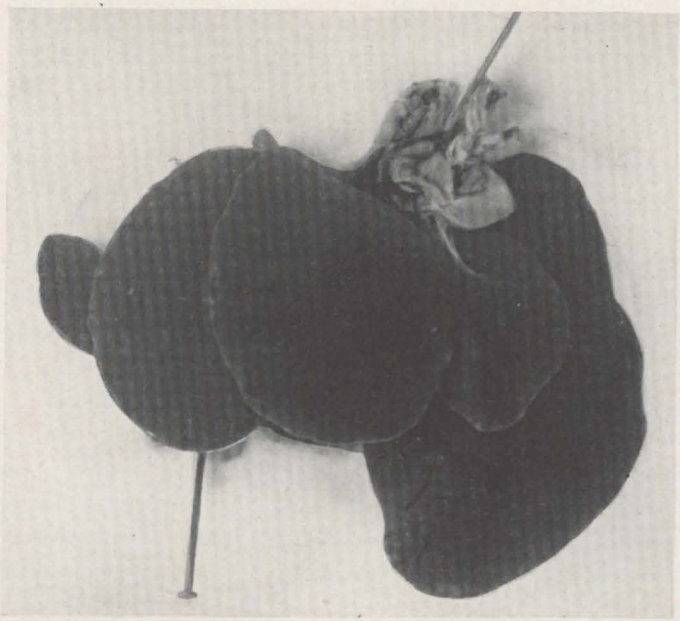

FIGURE 27