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PROGRESS REPORT

January 1973

MALARIA IMMUNITY AND VACCINATION

Director: Nelda E. Alger
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AID/csd 3689 Subcontract with U. of New Mexico

AGE AND PROTECTION

Protection tests completed to date by the intraperitoneal (IP) route show considerable variation from experiment to experiment with resultant difficulties in interpretation. We have usually used one age of mouse in any one experiment, however, two questions arise; one as to whether there is any difference in immunologic competence depending on the age at which injections were started and the second as to susceptibility to challenge at varying ages. Results (Table 1) indicate that there is no difference in the competence of the mouse to respond to the antigen, nor is there a difference in susceptibility to infection at challenge. Thus, we can rule out age as a complicating factor in interpreting our results. The variance seen here (40% to 90%) is usual in our tests by the IP route. It is to be hoped that our present intravenous (IV) series of tests will show less variance. Dr. R. Nussenzweig (personal communication) feels there is less variation by this route.

ROUTE OF INJECTION AND ADJUVANT

A combined experiment was designed to investigate the intramuscular (IM) vs. the IP route of injection, each route with and also without the adjuvant sodium alginate. Table 2 shows the results of this test. The IM route still appears unfavorable whether with or without the adjuvant since no protection was afforded. The procedure used necessitated the use of Clements medium for the first injection, but the results indicate that neither the adjuvant nor the Clements medium had any effect on the resultant protection, which varied from 50 - 90%, which is usual for this route.

These results confirm our previous findings (that the IM route of injection is poor for this system) and indicates that sodium alginate is not a useful adjuvant for our purposes.

VARIOUS TREATMENTS OF SPOROZOITES AND VARIABLE DOSAGE

Table 3 confirms our previous report that freeze-thawing does not appreciably affect protection. The results also confirm that seven injections of 25,000 sporozoites confers as good protection as larger numbers. It appears that so long as the minimum threshold of antigen is reached, larger doses are not helpful. In view of the difficulty encountered in obtaining sporozoite antigen this is encouraging. Once the best route of injection is established, it may well be possible to reduce the amount of antigen now required.

ROLE OF SALIVARY GLANDS IN PROTECTION

It is well known (see previous report) that insect hemolymph induces hypersensitivity. Since we feel that our previous experiments using cockroach material rule out non-specific type reactions as a mechanism of protection in this system, the mechanism of protection afforded is not clear. Our previously reported experiments with serotonin certainly suggest that hypersensitivity plays a role in this protection but how large a role is uncertain. Table 4 shows the results of an experiment designed to compare the hypersensitive reaction to hamster serum with that possibly induced by salivary glands. Sporozoites + serum + 3.7 salivary glands (average per injection per animal) resulted in a good protective response (19/20) whether with or without serotonin's partial blockage of the hypersensitive response. The addition of 70 salivary glands (groups 5 and 6) with or without serum did not show an appreciable difference (13/20) given the variability encountered in the system. Sporozoite-protected groups

with 3.6 salivary glands (average per injection per animal) resulted in 13/20 protected which also falls within this range. All serum controls with 3.7 glands showed "protected" animals (groups 11-14) but at low and questionable rates. While in groups 15 & 16, 70 salivary glands whether in medium 199 or serum gave good protection if serotonin was not used.

The results indicate that the sporozoite itself is a major component of the protective material and that some protection is afforded by a hypersensitive response to serum.

It is doubtful that serotonin would eliminate the hypersensitive response in all animals even though serotonin is a major component in the reaction. In Table 5 hamster serum alone (groups 3 and 7) protected 4/18 and 9/19 respectively. This protection was as good as the protection in Group 1 (sporozoites, serum and salivary glands). Hamster serum affords some protection 4/18) but rat serum does not (0/20 when used at the same dosage (groups 3 and 8).

It seems clear that hypersensitivity can protect an animal on challenge, but induction of hypersensitivity in individuals of a group is variable (Table 6). Further tests may help elucidate these problems.

Although the mechanism is not clear, results do indicate that at least 70 salivary glands per animal per injection are required for appreciable protection.

NORMAL MOSQUITO TISSUE

A rather large experiment utilizing the IM route of injection and sodium alginate vs. no sodium alginate served only to substantiate that this is a poor route of injection and a poor adjuvant.

FRACTIONATION AND PURIFICATION

Whole mosquitoes were ground in EDTA borate buffer in a Virtis mixer, fractionated through Sepharose 6B and the C & D peaks further fractionated through Biogel A 1.5. A peak designated D was administered 250 μg/inj/an in the same manner as the regular immunization procedures. No protection was found. The sporozoite control in this experiment showed 4/5 animals protected, the salivary gland control of the sporozoite injected animals (average 6.2 glands per animal per injection) showed 1/5 protected while 0/10 of the normal controls were protected. Since column chromatography is very time-consuming and considerable material is lost during manipulation, we have dropped this method for the present.

A different approach to fractionation has been started utilizing the Model L preparative centrifuge. The clear supernatant of normal mosquitoes has been fractionated by electrophoresis yielding at least 13 bands. Rabbit antiserum used against these fractions yielded a precipitin band in at least three areas. Each fraction will be collected for animal protection tests from both the preparative acrilimide gel and the pellets formed during centrifugation.

Distinct sporozoite bands did not show up in similar acrilimide preparation. Whether this was due to too little material or to the fact that the lines were obscured by the salivary gland material is unclear as yet.

BLOCKAGE OF THE MALARIAL CYCLE BY ANTIMOSQUITO ANTIBODY IN THE MOSQUITO BLOOD MEAL

The first half of this experiment was similar to an earlier experiment performed on the regular mouse colony. Of those mosquitoes which fed on mice, only 3-18

per cage survived out of approximately 150, while of those mosquitoes which fed on a rabbit, 109 survived. The average number of sporozoites per mosquito varied from 1.1×10^2 to 7.8×10^3 depending on the particular mouse on which the mosquitoes had fed. Only 2/17 normal mice became infected on challenge with these sporozoites. This suggests that they are not viable for the most part.

The second half of the experiment was undertaken with a colony of mosquitoes which has been maintained on mouse (rather than rabbit) blood. Some selection of the mosquitoes appear to have taken place since fewer mosquitoes now die in the first 24 hours after blood feeding than died previously in that period. The results are as yet incomplete but the sporozoites appear to be fairly infective (2/35 animals negative on day 8).

Table 1

Age and Protection

Age on first injection	25,000 sporozoites	Medium	Average no. salivary glands	Protected/Total
3 weeks	+	199	2.6	7/9
4 weeks	+	199	2.6	4/9
5 weeks	+	199	2.6	6/10
10 weeks	+	199	2.6	4/10
12 weeks	+	199	2.6	9/10
3 weeks	–	199	2.6	1/10
4 weeks	–	199	2.6	0/10
5 weeks	–	199	2.6	0/10
10 weeks	–	199	2.6	0/10
12 weeks	–	199	2.6	0/10
3 weeks	–	–	–	0/10
4 weeks	–	–	–	0/10
5 weeks	–	–	–	0/10
10 weeks	–	–	–	0/10
12 weeks	–	–	–	0/10

7 injections were given at two-week intervals. All injections were heated at 40-42°C for 60 minutes. Animals were challenged with 2,000 sporozoites three weeks following the final injection.

Table 2

Route of Injection and Adjuvant

Group	Number Sporozoites per Injection	Dissecting Medium	Allowed to sit at room temp. for 1 hour First Injection	Route of Injection	Sodium Alginate	Protected/ Total
1	25,000	C+199**	+	IM	+	0/10
2	25,000	C+199	+	IM	–	0/10
3	25,000	C+199	+	IP	+	5/10
4	25,000	C+199	+	IP	–	7/10
5	25,000	C+199	–	IP	–	9/10
6	25,000	199	–	IP	–	4/9
7*	–	C+199	+	IM	+	0/10
8*	–	C+199	+	IM	–	0/10
9*	–	C+199	+	IP	+	2/10
10*	–	C+199	+	IP	–	0/10
11*	–	C+199	–	IP	–	0/10
12*	–	199	–	IP	–	0/10
13	–	–	–	–	–	0/7

**Clements used for the first injection and medium 199 for injection 2-7. All injections heated 40-42°C for 60 minutes.

*Groups 7-12 represent the salivary gland control groups (average 3.7-3.9/ animal/injection).

Table 3
Freeze-thaw
Variable dose

Number Sporozoites* per Injection	Dissection Medium	Average No. Salivary Glands	Protected/Total
450,000	199	65.8	6/10
200,000	199	29.9	8/10
50,000	199	5.8	4/10
–	199	65.8	0/10
–	199	29.9	1/9
–	199	5.8	0/10
–	–	–	2/10

7 injections were given IP at two-week intervals and animals were challenged three weeks from the final injection.

*All injections were frozen in liquid nitrogen, thawed and refrozen three times.

TABLE 4
Hypersensitivity and Protection

Group	# Sporozoites/inj.	Dissecting Medium	Treatment	Serotonin	Average # Salivary Glands	Protected/Total
1	25,000	199	heat*	+	3.6	5/10
2	25,000	199	heat	--	3.6	8/10
3	25,000	serum**	heat	+	3.7	10/10
4	25,000	serum	heat	--	3.7	9/10
5	25,000	199	heat	--	70.0	6/10
6	25,000	serum	heat	--	70.0	7/10
Controls						
7	--	199	heat	+	3.6	1/10
8	--	199	no heat	+	3.6	0/10
9	--	199	heat	--	3.8	0/10
10	--	199	no heat	--	3.6	2/10
11	--	serum	heat	+	3.7	2/10
12	--	serum	no heat	+	3.7	4/10
13	--	serum	heat	--	3.7	3/10
14	--	serum	no heat	--	3.7	5/9
15	--	199	heat	--	70.0	9/10
16	--	serum	heat	--	70.0	6/9
17	--	--	--	+	--	1/9
18	--	--	--	--	--	2/10

7 injections were given IP at two-week intervals and animals were challenged with 2,000 sporozoites (in 0.05 hamster serum) 3-weeks following the final injection.

*Heat treatment was 40-42°C for 60 minutes.

**Hamster serum was as nearly as possible 0.05 ml/injection. The dilution for injection was made with medium 199.

Table 5

Group	Number Sporozoites per Injection	Dissecting Medium	Treatment	Average # Salivary Glands	Protected/Total
1	25,000	Hamster Serum 0.05/an/inj	heated	2.6	9/19
2	25,000	199	heated	1.8	6/17
3	–	Hamster Serum 0.025/an/inj	heated	–	4/18
4	–	Rat Serum 0.025/an/inj	heated	2.6	3/19
5	–	Hamster Serum 0.05/an/inj	heated	2.6	3/20
6	–	199	heated	1.8	0/20
7	–	Hamster Serum 0.05/an/inj	heated	–	9/19
8	–	Rat Serum 0.025/an/inj	heated	–	0/20
9	–	–	–	–	0/20

Only three injections were given IP at two-week intervals. Mice were challenged with 2,000 sporozoites in 0.05 serum diluted with 199.

Table 6

Role of Hypersensitivity in Protection

Number Sporozoites per Injection	Dissecting Medium	Treatment	Serotonin	Average # Salivary Glands	Protected/Total
—	Serum*	heat***	+	—	0/10
—	Serum	heat	—	—	5/9
25,000**	Serum	heat	—	3.2	9/10
—	Serum	heat	—	3.2	2/9
—	—	—	+	—	1/8
—	—	—	—	—	0/8

*7 injections were given IP at two-week intervals and animals were challenged with 2,000 sporozoites (in 0.05 hamster serum) three weeks following the final injection.

**Sporozoites were dissected in 0.05 ml of hamster serum which was diluted with 199 for injection.

***Heat treatment was 40-42°C for 60 minutes.

ELECTRON MICROSCOPY

Various crude fractions of mosquito tissue prepared by the French pressure cell disintegration method have been processed for electron microscopic studies. Unfortunately to date these fractions have not been observed in the electron microscope due to a major breakdown in the instrument. The electron microscope was not used in the early summer because it was not under contract for servicing. Some time elapsed before the servicing of the microscope was re-established on a contractual basis. Once this was in operation again, it was discovered that the entire gun assembly was malfunctioning and would have to be replaced. The parts needed were ordered from Japan and, needless to say, this took time. Although the microscope has been "repaired" it still is not in good functioning order due to a lack of sufficient illumination as well as instability of the electron beam. Hopefully those problems will be resolved shortly.

TISSUE SECTION ANALYSIS OF RHESUS MONKEYS USED IN *PLASMODIUM KNOWLESI* VACCINATION EXPERIMENTS

General Comments

An extensive study and comparison of various tissue sections of the rhesus monkeys used in *P. knowlesi* vaccination experiments revealed some interesting and useful information. The insight and knowledge obtained from these observations would have been even more valuable if detailed data were available as to the exact treatment of the tissues at the time of death or sacrifice of the monkeys. Lack of this information prevented a comparison of tissues of the experimental animals with those of normal and untreated controls which would have been most invaluable. Because such data are not available comments on the tissue sections that have been analyzed have been limited by necessity to the distribution of bacterial contamination, malarial pigment and the actual presence of *P. knowlesi* in the red blood cell (RBC) or other cell types.

Observations made on the different tissue sections will be described separately for each animal and the results summarized in Table II. Roughly, three to four slides were prepared for each tissue. Each slide was carefully studied at least three to four different times so as to glean as much information from these experiments as possible. Where questions arose as to interpretation some slides were read as many as six times with careful notes taken each time.

SPECIFIC OBSERVATIONS ON VACCINATED MONKEYS

MONKEY #1484

Liver:

Only a few typical malarial pigment granules of P. knowlesi were seen in the liver sections. The granules were uniform in size and density and were located primarily within, what was believed to be, the phagocytic cells of the liver. At no time was the parasite P. knowlesi observed in the parenchymal cells. It is also believed that there were no parasites within the RBCs. A contaminating brown pigment in the form of large and small spheres was noted. These spheres were seen most often within nucleated cells, but on occasion were also observed free in the tissue. The spheres were not uniformly distributed throughout the sections. In addition small structures resembling in appearance rod- and sphere-shaped bacteria were noted within cells, free in the tissue and in the blood vessels. In fact, some blood vessels appeared to be packed with these unidentified bacteria. It is also for this reason that one cannot state with great assurance that P. knowlesi were not present within the RBC because of the excessive bacterial contamination. The bacteria approximate in size the identifying characteristics of the P. knowlesi nuclear cluster. In general, the liver tissue was very granular with large areas of cell damage. Many cells were highly

vacuolated, as if undergoing fatty degeneration, which is also known to occur in animals infected with P. knowlesi. In this case, however, such a conclusion is unwarranted because similar damage may also be encountered in poor tissue preservation. Blood within the vessels gave the impression of being coagulated. Only in very rare instances were individual RBCs encountered.

Spleen:

As in the liver, so in the spleen, only an occasional malarial pigment granule was encountered. Likewise, it appeared that no P. knowlesi had parasitized any RBCs. Large areas of what appeared to be coagulated blood were seen in the red pulp of the spleen. Again, as in the liver, the spleen tissue was overloaded with bacterial organisms.

Kidney:

No sections were cut.

Cerebrum:

Neither malarial pigment nor parasitized RBC were ever observed in the brain sections. The tissue was extremely granular and shrinkage artifacts were especially noted in the areas surrounding blood vessels.

MONKEY #1489

Liver:

The liver sections of this monkey gave a slightly different picture than those of animal 1484. It seems that there was much less bacterial contamination and an occasional RBC containing the characteristic P. knowlesi form was observed. The amount of malarial pigment within the liver cells appeared to be the same. The parenchymal cells themselves were highly vacuolated and granular. Blood within the vessels seemed coagulated.

Spleen:

The spleen sections, unlike the liver, contained slightly more malarial pigment as well as more parasitized RBCs. The same type of bacterial contamination was seen in the spleen sections of this monkey as was seen in animal 1484, but in this case it was much less. In addition, in favorable sections, numerous eosinophils were encountered. The tissue was highly reticulated and large areas of coagulated blood were observed in the red pulp.

Kidney:

The kidney tissue, as shown by the sections, was extensively damaged, especially the tubular epithelium. This type of damage also may occur due to P. knowlesi infection, but in this case such conclusions cannot be made because of the unknown factors involved in the tissue preservation technique. Also, the parasitemia of this monkey seemed to be too low to induce such damage. Although malarial pigment was not observed in the kidney sections, parasitized RBCs were seen in the glomerular capillaries as well as other blood vessels. In many glomerulae the Bowman's space was clear and devoid of any matter, while in others an amorphous material, similar in appearance to the damaged tissue outside the glomerulus, reminiscent of connective tissue, was present.

Cerebrum:

Malarial pigment granules were not seen, but parasitized erythrocytes were frequently encountered in the cerebral sections. The tissue was extremely vacuolated and lacy and for the most part only cell nuclei of nerve cells were evident. Shrinkage artifact was quite pronounced.

MONKEY #1483

Liver

An increased amount of malarial pigment was seen in the liver sections of this monkey as compared to animals

1484 and 1489. Bacterial invasion of nucleated cells, as well as bacteria, free in the blood vessels, was especially severe. The vascular bacteria made it extremely difficult to identify parasitized RBCs with any great assurance, but the impression was that parasitized RBCs were present. Relatively many immature erythocytes were noted in the liver sections of this monkey as compared to animals 1484 and 1489. Although many large areas resembling coagulated blood were present, the tissue seemed fairly intact.

Spleen:

Much more malarial pigment was seen in the spleen cells than in the liver, but the impression of parasitized RBCs remained the same. Bacterial contamination was especially high. Extended areas of coagulated blood were noted throughout the red pulp and the immediate areas surrounding the white pulp.

Kidney:

Only a few malarial pigment granules were observed in the kidney sections. An occasional parasitized RBC was seen within the glomerular capillary network as well as in the cortical and medullary vessels. The Bowman's space of some glomerulae was clear while in others it was filled with some unidentified granular material. In general the tissue appeared fairly intact.

Cerebrum:

Only a few grains of malarial pigment were seen in what was interpreted as parasitized RBCs. Identification of P. knowlesi, however, was hindered by the excessive number of bacterial organisms as it was in Monkey 1484. The blood vessels were solid with packed RBCs rather than

containing coagulated blood as was the case in some of the other tissues of monkeys 1484 and 1489. The difference between the terms packed and coagulated is in the appearance of the RBCs. Where the term packed is used, individual RBC can be identified even though the blood vessels are solidly occluded. Coagulated, on the other hand, implies that a solid mass is present without any noticeable RBC outlines. The cerebrum, as was the case with other tissues of Monkey 1483, seemed quite well preserved.

SPECIFIC OBSERVATIONS OF CONTROL MONKEYS

MONKEY 1476

Liver

Large numbers of malarial granules were distributed uniformly throughout the liver. Most often the pigment was deposited within the parenchymal cells. For some reason the RBCs did not stain in these sections and therefore gave the impression that great numbers of P. knowlesi were free within open spaces of the liver. The tissue as a whole was very poorly preserved in that it was very granular and the parenchymal cells highly vacuolated. The sections appeared to be free of any bacterial organisms, but due to the heavy deposition of malarial pigment the possibility of their presence cannot be excluded entirely.

Spleen:

Spleen sections of Monkey 1476 were most difficult to study because of the excessive accumulation of malarial pigment. Pigmentation was so pronounced that it actually hindered individual cell identification of the reticular tissue as well as of the RBCs. However, when mature RBCs were identified, more often than not, they contained P. knowlesi. The blood for the most part was coagulated and where individual RBCs were seen, they appeared highly distorted.

Kidney:

Some malarial pigment was seen in the kidney, but this usually was localized within the mature RBC. These RBCs were observed in the blood vessels as well as the glomerular capillaries. Although the tissue appeared greatly damaged, the damage was different from the type seen in Monkey 1484. In most instances the Bowman's space was open and clear even when its glomerulus was loaded with clumped parasitized RBC.

Cortex:

A small amount of malarial pigment was seen within the blood vessels. More often than not blood within the vessels of the cortex appeared coagulated without clearly identifiable RBCs. Many P. knowlesi were seen throughout this coagulum as identified by the characteristic quadruple nucleation of the parasite.

MONKEY 1477

Liver:

Although the liver sections of this monkey closely resembled the sections of animal 1476, there was somewhat less malarial pigment in the former. On the other hand, there were more parasitized RBCs. Many immature RBCs were also noted. The parenchymal cells were highly vacuolated and granular. The tissue was very poorly preserved and stained. There did not seem to be any bacterial contamination within the sections.

Spleen:

The spleen slides of this control monkey were very similar to the spleen sections of animal 1476. An excessive amount of malarial pigment as well as parasitized RBCs were seen throughout the tissue. Large areas of coagulated blood were present in the red pulp.

Kidney:

Although very little malarial pigment was noted in the kidney, the capillaries of the glomerulus, and the kidney in general, were severely packed with P. knowlesi parasitized RBCs. The vessels in general appeared packed with P. knowlesi parasitized RBCs. The Bowman's space was fairly clean of any precipitate. The tissue was greatly damaged and granular, probably due to poor preservation.

Cortex:

Blood vessels within the cortex were severely distorted and shrunken and contained coagulated blood. A large number of P. knowlesi were observed in the vessels, but hardly any malarial pigment was seen.

MONKEY 1479

Liver:

Even though an excessive number of P. knowlesi parasitized RBCs were observed in the liver sections, only an average amount of malarial pigmentation was present. The parenchymal cells were vacuolated and granular. No immature RBCs were noted. Blood was in the form of a coagulum, but individual RBCs were also seen occasionally. Bacterial contamination seemed to be lacking.

Spleen:

The spleen sections showed a pronounced degree of malarial pigmentation as well as an excessive amount of parasitized RBCs. Blood in the red pulp was coagulated, but individual RBCs were also seen. Many RBCs, parasitized and unparasitized, were noted in the white pulp likewise.

Kidney:

The kidney sections contained many parasitized RBCs. The Bowman's space was filled with a precipitate which was also seen in the tubules. A similar substance was

similarly noted in the liver. The tissue appeared extremely poorly preserved.

Cortex:

An excessive number of P. knowlesi parasitized RBCs were observed in the blood vessels. The parasites were so heavily pigmented that in some spots where the RBCs were packed they appeared as areas of pigment storage. Individual RBCs, as well as coagulated blood, were present in the blood vessels, which in turn appeared shrunken. There was a fibrous precipitate in the blood vessels where no individual RBCs were observed.

MONKEY 1480

Liver:

Malarial pigment was distributed uniformly throughout the liver sections. A large number of parasitized RBCs were present. A fibrous precipitate, similar to that observed in the kidney and the cortex sections of Monkey 1479 was seen in the blood vessels. The tissue was very granular and poorly preserved. No apparent bacteremia was evident.

Spleen:

The spleen sections of this monkey were very similar to those of animal 1479.

Kidney:

The kidney sections resembled very closely those of Monkey 1479 except that in this case the Bowman's space was comparatively lacking in the fibrous precipitate. The fibrous and granular precipitates, however, were seen in the collecting tubules and blood vessels.

Cortex:

Sections of the cortex contained many blood vessels which appeared solid with a precipitate. Many P. knowlesi

were seen within the vessels. Some of the parasites stained blue rather than their characteristic color.

CONCLUSION:

Even though the experimental results suggest that some of the vaccinated monkeys were protected, the true source of the protection is still obscure because of the severe bacteremia of the vaccinated animals. The influence of the bacteria on the immune mechanism of the monkey cannot be ignored. Bacteria and their products, up to a point, may have contributed to the adjuvanting effect of the vaccinating material. Interpretation of the experimental results are complicated in that only those monkeys which had been vaccinated contracted the bacterial infection. This suggests, of course, that the source of the vaccinating material was contaminated with live bacteria. An additional effect that the bacteria may have had on P. knowlesi is that they may have produced an unfavorable environment for the malaria parasite, either by competing for certain essential substances of the host, or by producing substances toxic to the monkeys as well as to P. knowlesi and thereby suppressing its usual establishment of P. knowlesi within the animal.

TABLE I. Explanation of the difference between the various groups.*

GROUP	ANIMAL	TREATMENT AND	ROUTE OF INJECTION
I	1481, 1486, 1493	1ml regular Ag + Freund's Complete	Intra muscular
II	1484, 1489, 1491	Pk. I material + " "	" "
III	1483, 1492, 1497	Pk. II " " " "	" "
IV	1476, 1477	1ml regular Ag, no adjuvant	" "
V	1479, 1480	1ml Freund's Complete, no Ag	" "

*Tissue sections were prepared only of those animals whose numbers have been underlined. Of these animals, slides were made of the liver, spleen, kidney and brain. Only specimen 1484 was lacking in kidney sections.

TABLE II. Qualitative distribution of malarial pigment and P. knowlesi parasitized RBC in the various tissue sections studied.°*

GROUP	ANIMAL	BACTERIAL CONTAMIN. **	TISSUE SECTIONS							
			LIVER		SPLEEN		KIDNEY		BRAIN	
			Pgmt.	P. knwl	Pgmt.	P. knwl.	Pgmt.	P. knwl.	Pgmt.	P. Knwl
I		N O	S L I	D E S						
II	1484	5+	+	?-	+	?-	NO	SLIDE	-	?-
	1489	2+	+	+	2+	2+	-	2+	-	2+
III	1483	5+	2+	?+	3+	?+	+	?+	+	?+
IV	1476	?-	4+	4+	5+	5+	3+	5+	+	5+
	1477	?-	3+	5+	5+	5+	2+	5+	+	5+
V	1479	?-	3+	5+	5+	5+	3+	5+	+	5+
	1480	?-	4+	5+	5+	5+	3+	5+	+	5+

° = See Table 1 for experimental treatment of groups and animals.

* = An arbitrary system of five points was chosen

5+ = Highest number observed.

?- = Not sure as to the accuracy of the observation, but the impression was distinctly negative.

?+ = Not sure as to the accuracy of the observation, but the impression was distinctly positive.

- = None observed.

? = Any bacterial contamination, if present, would not have been noticed due to excessive malarial pigment deposition.

** = A general impression of the bacteremia observed in all of the tissues as well as the blood (in blood vessels).