left filling such a meaningless territory as this, but are modified in the way that has been noted until they correspond with the body as the sufferer perceives it.

Summary

Deep pain, unlike superficial sensation, comes from parts of the body that are unperceived and to which, it is argued, no sensation can be localized. All sensations are normally felt somewhere, and deep pain must therefore be an alloaesthesia. It is suggested that these "pains from nowhere" are projected into the perceived parts of the body. They have a segmental basis derived from the fixed structures of the cortex. Their locality is further defined within these crude limits according to the individual characteristics of the body image. The sensation of deep pain does not depend on any peripheral change in the part where it is felt. But its location does depend on the normal stimuli coming from the part, for without these the place would remain unperceived.

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THE PRE-ERYTHROCYTIC STAGE OF MAMMALIAN MALARIA

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Since the discovery of the part played by the mosquito in the transmission of malaria there has remained a gap in our knowledge of the cycle of events. This has been our complete ignorance of what happens to the sporozoites injected by the mosquito during the period between its bite and the appearance of erythrocytic parasites in the circulating blood. Schaudinn's (1902) observation of the sporozoite entering a red cell has never been repeated and has long been suspect, and the most generally held theory of recent years has been that some form of development takes place in the internal organs—probably in the reticulo-endothelial system—before the parasites enter the red cells of the circulation at the end of the incubation period. This interval may be considered to be a period of about ten days.

The curtain was first really lifted in the case of avian malaria when James and Tate (1937) described the exo-erythrocytic cycle in *Plasmodium gallinaceum*, but the developments following the introduction of sporozoites by the mosquito remained a mystery. The next step was the discovery by Mudrow (1940) and Shortt, Menon, and Iyer (1940) of developmental forms of *P. gallinaceum* in the incubation period; these were schizogony forms in reticulo-endothelial cells. Reichenow and Mudrow (1943) next gave a detailed description of the forms found during the incubation period in infections with *P. relictum*, and Huff and Coulston (1944) independently described in even greater detail the course of events in *P. gallinaceum*.

All this work led to the belief that some parallel development must take place in the case of mammalian (including human) malaria, but all attempts to demonstrate this proved unsuccessful. Efforts to discover the incubation period stage of mammalian malaria—i.e., the hypothetical pre-erythrocytic stage—were given a stimulus by the work of Fairley (1945) at Cairns, Australia, during the recent world war. Fairley and his co-workers showed that during the biting act of the malaria-infected mosquito, and for about half an hour afterwards, blood inoculated from the bitten person into a volunteer produced an infection. After this period and until the appearance of the parasites in the circulating blood even large quantities of blood from the volunteer produced no infection in another individual.

From these results and others obtained in the field of chemotherapy—for example, Davey (1946)—it was evident that shortly after the introduction of sporozoites they disappear from the circulation and the further development during the incubation period takes place in some protected site outside the general circulation.

Hitherto there has been little to support this hypothesis in the form of actual findings in mammalian malaria. Raffaele (1937) described bodies found in smears of human bone marrow and considered by him to represent stages in the development of sporozoites. Somewhat similar observations have been made by other workers in this field up to the present date, but none of these have been confirmed or have even received much support.

Large-scale experiments with *P. cynomolgi* carried out by the Mammalian Malaria Inquiry under the Director, Central Research Institute, Kasauli (1946), and similar investigations by Huff and Coulston (1947), have yielded negative results.

In the Department of Parasitology at the London School of Hygiene and Tropical Medicine investigations to discover the pre-erythrocytic stage of *P. cynomolgi* in the rhesus monkey have been in progress since 1945, and the most recent experiments have at last resulted in the discovery of this stage, as briefly reported by Shortt and Garnham (1948).

Description of Experiment

We do not propose to give the details of all our work, but will confine ourselves to a description of our most recent successful experiment. More than 1,000 *Anopheles maculipennis atroparvus* bred in the laboratory were fed on a monkey showing mature gametocytes in the peripheral blood. The mosquitoes were subsequently fed on another infected monkey and were given a third feed on the original animal. The interval between the first and third feeds was eleven days. The mosquitoes were maintained at 26° C. in a relative humidity of approximately 80%.

Ten days after the last infective feed 20 mosquitoes were dissected and without exception proved infected, most of them with extremely numerous sporozoites in the glands.

On that day the survivors, 576 in number, were given the opportunity to feed upon a clean rhesus monkey ; over 500 did so. The entire batch of mosquitoes was then ground up in a mortar in 10 ml. of heparinized monkey plasma diluted with normal saline solution. Half the suspension was inoculated intraperitoneally into the same monkey and the other half into the thigh muscles of both sides. The suspension showed an average of 5 sporozoites per field of the 1/12 oil-immersion objective.

The monkey was sacrificed seven days later and a very complete necropsy was conducted.

The tissues given in the list which follows were taken for examination either in smears or in sections, or both : Spleen, liver, kidney, suprarenal gland, pancreas, small

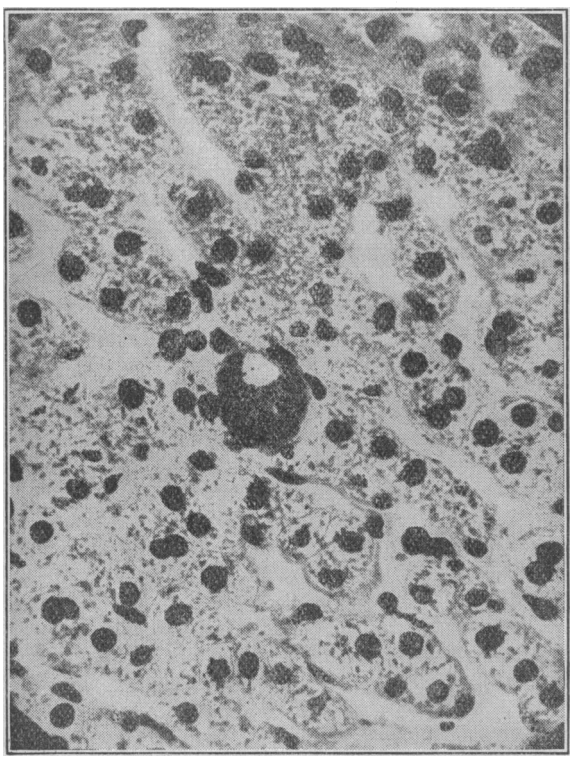

FIG. 1.—Section of liver showing schizont of *P. cynomolgi*, containing well-marked vacuole. Mod. Giemsa stain (×500).

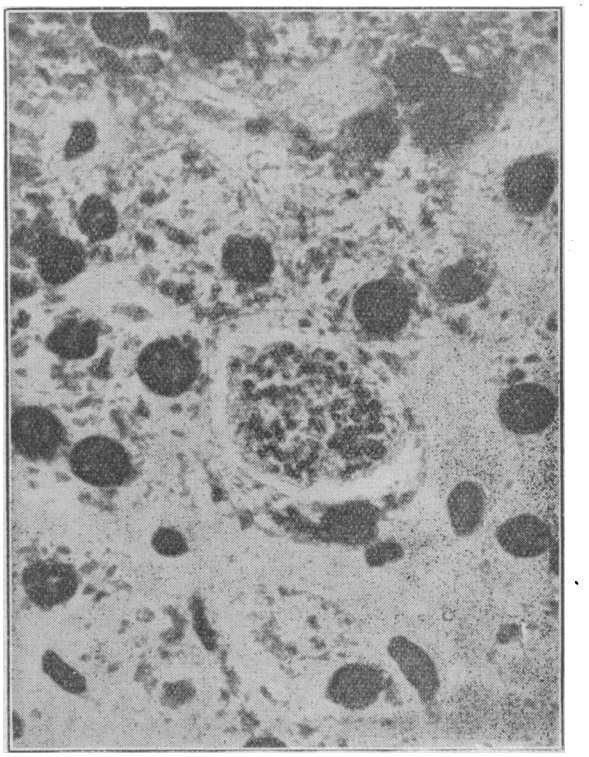

FIG. 2.—Section of liver showing schizont of *P. cynomolgi*, with numerous masses of chromatin. Mod. Giemsa stain (×1,000).

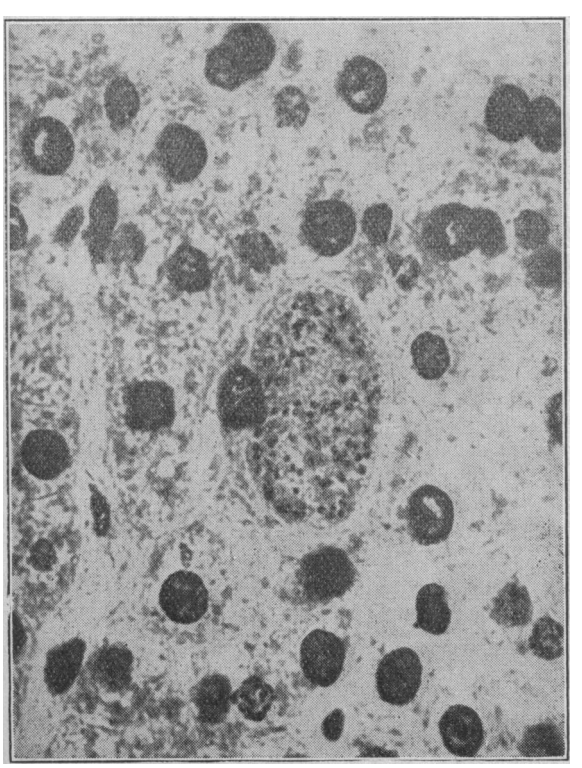

FIG. 3.—Section of liver showing oval schizont of *P. cynomolgi*. Mod. Giemsa stain (×1,000).

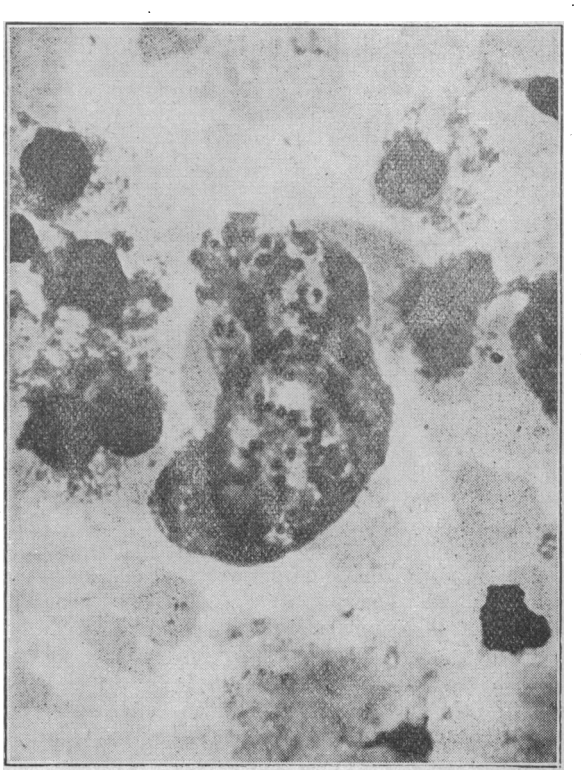

FIG. 4.—Schizont of *P. cynomolgi* found in an "impression smear" of liver. Giemsa stain (×1,000).

intestine, lymph glands from various parts of the abdomen, aorta, inferior vena cava, peritoneal exudate, peritoneum, lungs, heart, thoracic glands, bone marrow, brain, leg muscles, stretch preparations of pia mater and omentum.

The fixatives used were Zenker, Carnoy, Flemming, and 10% formol-saline. Suspensions in citrated saline of the following tissues, in the amounts stated, were inoculated into clean monkeys both intraperitoneally and intramuscularly in each case: liver, 10 ml.; spleen, 8 ml.; brain, 7 ml.; lung, 12 ml.; kidney, 8 ml.; peritoneal fluid, 4 ml.; and heart blood, 7 ml. All the monkeys have remained negative for a month.

The slides of material were stained with haematoxylin and eosin or Giemsa, using the modification of McNamara's stain described by Shortt and Cooper (in the press). The latter unquestionably gives the most brilliant results, and the description given below applies to parasites stained by that method.

Pre-erythrocytic Stage on Seventh Day of Incubation Period

Up to the time of writing we have not had the opportunity of examining thoroughly all the tissues taken, but a rapid survey has resulted in the finding of parasites in the liver only. In a section of the liver examined with the 2/3 objective, small areas of blue colour and ovoid shape are seen very thinly scattered throughout the section (Fig. 1). In one typical section with an area of 90 sq. mm. there were 36 such areas. The ovoid shape is not invariable, and some parasites may show minor indentations, whilst in a few cases actual blunt pseudopodic arms exist. The parasites measured an average of 26μ in the longest diameter, but larger forms up to 30μ or more occur.

When examined under high power these blue areas are seen to be plasmodial masses undergoing schizogony (Figs. 2 and 3). The cytoplasm stains a cobalt blue and has an opaque semi-reticulated appearance, while the particles of chromatin stain a magenta colour. In the majority of the parasites there is no evident condensation of cytoplasm around the chromatin masses, but in a few cases in sections cut at 2μ thickness there is a distinct indication of this process which would result in the formation of merozoites. We have seen parasites in the circulating blood on the ninth day after infection; it is therefore evident that merozoite production must have taken place about the eighth day and the forms described above would be the first stages in the process. For the same reason we conclude that the majority of the forms in the liver are nearly mature and at the stage immediately preceding merozoite formation.

In a considerable number of schizonts there appear one, two, or even multiple vacuoles (Fig. 1) with sharply cut outlines. These tend to be smaller the more numerous they are. It should be mentioned that at no stage is any pigment to be seen in the parasite.

It is very difficult to be certain of the number of particles of chromatin present, especially if the counting is performed on serial sections, because parts of the same fragment may be counted twice, and for this reason we have based our estimate on schizonts seen in an impression smear (Fig. 4). The number was estimated to be between 200 and 300 in a single schizont.

As regards the relationship of the parasite to the liver tissue we do not at present feel inclined to be dogmatic, and a final opinion can be formed only when younger stages of the parasite have been examined. The general impression gained by us, however, is that the parasites are originally contained in the parenchyma cells, and this opinion is strengthened by the appearance in sections stained by Gömöri's stain to show the reticular fibres. In a monkey sacrificed on the sixth day the parasites in the liver exhibited few, if any, differences from the seventh day forms.

Discussion

The importance of this discovery lies in the fact that the resemblance of *P. cynomolgi* to *P. vivax* of human malaria is so close that the findings here described will almost certainly be applicable to the human parasite and, therefore, that the liver is the most likely site for the human pre-erythrocytic forms.

Until we have seen the earlier pre-erythrocytic stages of *P. cynomolgi* we feel that we are hardly in a position to discuss the relationship of the forms found by us to comparable stages of other pigment-producing blood parasites. On the other hand it may be noted that there is a superficial resemblance between the liver schizonts of *P. cynomolgi* and the tissue phase of *P. gallinaceum*, where, however, the merozoites would appear to be more numerous. There is an even closer resemblance to the early exo-erythrocytic stage of *Hepatocystes* (*Plasmodium*) *kochi* (Garnham, in press), although it must be remembered that the fully developed stage of the latter in the liver measures 2 mm. in diameter—i.e., at least 80 times the size of the mature *P. cynomolgi* schizont.

The failure hitherto to find pre-erythrocytic stages of mammalian malaria may be attributed to certain factors: (*a*) the dilution factor, which necessitates an enormous dosage of sporozoites if the developmental forms are to be readily found; (*b*) examination of smears has been more intensive than of sections; in smears the parasites are less readily found. The fact that exo-erythrocytic forms had not been found in mammalian malaria may be due to their possibly evanescent nature. It seems likely that the majority, at least, disappear with the establishment of the erythrocytic cycle.

We wish to acknowledge the great help we received in this investigation from our laboratory staff. Mr. W. Cooper with his great technical skill and experience has been invaluable and was ably seconded by Mr. E. Blackie, while Miss J. Stedman gave valuable assistance in mosquito technique. We are also indebted to Mr. W. Alves, B.A., for assistance in the critical necropsy on the first monkey in which we found pre-erythrocytic forms.

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Nurses, medical auxiliaries, and certain other hospital or allied workers are excepted from the Control of Engagement Order, 1947. Hospitals and employing authorities may therefore advertise for and engage such staff without reference to the Ministry of Labour and National Service. Those excepted include the following: State-registered nurses, student nurses, health visitors, midwives, and certain unqualified persons on nursing duties, such as nursing auxiliaries. Domestic workers in hospitals are covered by the Order, under which hospital domestic work is regarded as essential work. Full details are contained in the Ministry of Health circular 1/48, dated Jan. 15.