LETTERS TO THE EDITORS

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Pre-erythrocytic Stage in Mammalian Malaria Parasites

THIS laboratory, in common with many others throughout the world, has for years been working on the life-cycle of malaria parasites in the vertebrate host. The most important details have already been elucidated so far as avian malaria is concerned, but the development of the sporozoites in the prepatent stage in mammalian malaria has remained obscure in spite of the most painstaking and meticulous work in many centres of malaria research.

The latest results obtained by us in this laboratory in the case of monkey malaria, *Plasmodium cynomolgi*, have been striking and, we think, unequivocal. The briefest details of these experiments are as follows.

Anopheles maculipennis, in large numbers, were infected by feeding on a rhesus monkey carrying gametocytes of *Plasmodium cynomolgi*. These mosquitoes, when at the infective stage, with large numbers of sporozoites in the salivary glands, were fed upon a clean rhesus monkey. The total number of anopheles was then ground up in a mortar in heparinized monkey plasma, diluted with normal saline solution, and half the resulting suspension inoculated intra-peritoneally and the other half intra-muscularly into the same clean rhesus monkey.

The latter was sacrificed seven days later and various internal organs were taken for examination by smears and in sections. Material was also inoculated into clean monkeys.

This material is now being examined; but the first results obtained are considered worthy of recording here.

In sections of the liver there appeared numerous parasites in the form of plasmodial masses undergoing schizogony. These measure about 25 μ–30 μ in the largest diameter. The masses are clear cut and are scattered through the liver tissue. In sections stained by Giemsa stain, the cytoplasm is a deep blue colour and the discrete particles of chromatin are a magenta colour, both being typical of the usual reaction of protozoal blood parasites with this stain.

Similar results have been obtained in the case of another monkey sacrificed on the sixth day of the incubation period. Further investigation is proceeding.

The importance of these results seems to us to lie in the fact that *P. cynomolgi* is a parasite very closely akin to *P. vivax* of human malaria, and it is reasonable to suppose that the human parasite will show similar developmental changes in the incubation period of an infection.

These results appear to confirm the almost universal belief in the existence of a pre-erythrocytic stage in the mammalian malaria parasites.

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Action of Nitrogen Trichloride on Proteins: Production of Toxic Derivative

FOLLOWING Mellanby's discovery[1] that 'Agenized' wheaten flour, when fed to dogs, produces hysteria, it was shown in a first paper[2] from these laboratories that the syndrome is not due to a nutritional deficiency but to the effect of a toxic substance produced by the action of nitrogen trichloride (the main constituent of commercial 'Agene') upon the wheat protein. Furthermore, nitrogen trichloride can react with other proteins such as casein and zein to produce a similar substance. The simplest conception, somewhat similar to that advanced by Dixon and Needham[3] for the action of mustard gas upon tissues, is that a reactive protein contains a number of centres, *C*, probably of several types, all of which react with nitrogen trichloride; but of these centres only one type, *E*, which may occur at several places in the molecule, is essential for the production of the toxic substance. As this can be produced by the complexes of nitrogen trichloride with wheat protein, casein, zein, egg albumin, hæmoglobin, rice protein and probably many other proteins, it is obvious that the *E* centre is common to most proteins. On the other hand, we have prepared two proteins (arachin and kerateine[4,5]) which absorb nitrogen trichloride to form complexes having no significant toxic effect, while gelatin is in much the same category.

The actual chemical interaction between nitrogen trichloride and *E* at the point of attack can involve only small molecular groupings. Nevertheless, it is almost certain that *E* must be part of a larger unit of form —*A*—*E*—*B*— contained within the protein molecule. The simplest form of unit is an individual amino-acid residue, but our failure to produce canine hysteria after treatment with nitrogen trichloride of the mixture of amino-acids obtained on acid hydrolysis of casein suggests that the unit is larger than a single amino-acid. One such unit, strepogenin, has been recognized as common to several proteins[6], but this particular unit cannot be involved in the toxic substance since it is absent in egg albumin.

If the active *E* centre is a portion of a particular amino-acid, it is necessary first to identify the acid and then to ascertain the nature of any environmental specific groupings necessary for the production of the full toxic effect. Our work has been confined chiefly to investigation of the chemical nature of the reactive *E* grouping.

The facile production of hysteria by the zein complex appears to eliminate lysine, tryptophane and glycine both as *E* centres and as necessary concomitant groups. Similarly, the harmless nature of the kerateine complex with its abundance of cysteine (11–12 per cent) and the marked toxicity of the casein complex with its relatively low cysteine content suggests that this amino-acid can also be ruled out, despite the suggestion of other workers[7].

Recent evidence indicates that the feeble toxicity of a mixture of gelatin and maize starch after treatment with nitrogen trichloride is due partly to the small amount of protein, about 0·4 per cent, in this starch. Gelatin is therefore a useful standard, and a comparison of its composition with that of the highly reactive casein and zein suggests that one or more of the following amino-acids are associated with the *E* centre: methionine, glutamic acid, tyrosine, phenyl-alanine, threonine, leucine and *iso*-leucine.

We have given some attention to the conditions under which nitrogen trichloride reacts with proteins

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