Problems connected with the proposal to supplement alcoholic beverages and saccharine aerated water ("soft drinks") with choline or its precursors are mentioned.

Our findings are, of course, not necessarily applicable to alcoholism in human subjects. If they should prove to be so, it is increasingly obvious that individuals who habitually consume large amounts of alcohol or sugar lack adequate amounts of the lipotropic agents as well as of other dietary essentials.

The classical hepatic lesions (fatty changes and fibrosis) associated with alcoholism in human subjects, the first descriptions of which are mentioned in our introduction, may prove to be due specifically to a lack of the lipotropic agents.

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REFERENCES

- Addison, T. (1836). *Guy's Hosp. Rep.*, **1**, 476.
 Ashworth, C. T. (1947). *Proc. Soc. exp. Biol.*, N.Y., **66**, 382.
 Best, C. H., Lucas, C. C., Patterson, J. M., and Ridout, J. H. (1946). *Biochem. J.*, **40**, 368.
 Beveridge, J. M. R., and Lucas, C. C. (1945). *J. biol. Chem.*, **157**, 311.
 Chaikoff, I. L., and Connor, C. L. (1940). *Proc. Soc. exp. Biol.*, N.Y., **43**, 638.
 ——— and Biskind, G. R. (1938). *Amer. J. Path.*, **14**, 101.
 Connor, C. L., and Chaikoff, I. L. (1938). *Proc. Soc. exp. Biol.*, N.Y., **39**, 356.
 Daft, F. S., Sebrell, W. H., and Lillie, R. D. (1941). *Ibid.*, **48**, 228.
 Duchek, A. (1853). *Prag. Vjschr.*, **3**, 104. Cited by Ruge (1870).
 Himsworth, H. P. (1948). *Sci. Progr.*, **144**, 577.
 Huss, Magnus (1852). *Alcoholismus chronicus*. Leipzig and Stockholm. Cited by Ruge (1870).
 Kremiansky, J. (1868). *Virchows Arch.*, **42**, 340.
 Lillie, R. D., Daft, F. S., and Sebrell, W. H. (1941). *Publ. Hlth Rep.*, Wash., **56**, 1255.
 ——— Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V. (1942). *Ibid.*, **57**, 502.
 Lowry, J. V., Daft, F. S., Sebrell, W. H., Ashburn, L. L., and Lillie, R. D. (1941). *Ibid.*, **56**, 2216.
 ——— Ashburn, L. L., Daft, F. S., and Sebrell, W. H. (1942). *Quart. J. Stud. Alcohol*, **3**, 168.
 Mitchell, H. H. (1935). *J. Nutrit.*, **10**, 311.
 ——— and Curzon, E. G. (1940). *Quart. J. Stud. Alcohol*, **1**, 227.
 Moon, V. H. (1934). *Arch. Path.*, **18**, 381.
 Patek, A. J. (1947). *J. Mt Sinai Hosp.*, **14**, 1.
 Rokitsansky, C. von (1849). *A Manual of Pathological Anatomy*, **2**, 145. Sydenham Society, London.
 Ruge, P. (1870). *Virchows Arch.*, **49**, 252.

THE PRE-ERYTHROCYTIC STAGE OF PLASMODIUM FALCIPARUM

A PRELIMINARY NOTE

BY

H. E. SHORTT, C.I.E., M.D., D.Sc., D.T.M.&H.

N. HAMILTON FAIRLEY, C.B.E., F.R.S.

G. COVELL, C.I.E., M.D., D.T.M.&H., D.P.H.

P. G. SHUTE

AND

P. C. C. GARNHAM, M.D., D.P.H.

The demonstration of a pre-erythrocytic cycle in mammalian malaria as it exists in the monkey malaria parasite, *Plasmodium cynomolgi* (Shortt and Garnham, 1948), led to the deduction that a similar cycle would be found in the case of human malaria caused by the parasite *P. vivax*. This followed from the close similarity of the two parasites, and the assumption was quickly proved experimentally by the discovery of the pre-erythrocytic cycle in a human volunteer (Shortt, Garnham, Covell, and Shute, 1948). In both cases the pre-erythrocytic cycle was shown to take place in the parenchyma cells of the liver.

It was subsequently demonstrated that this cycle persisted in the liver after the establishment of the infection in the

peripheral blood and that it was the probable cause of relapses in benign tertian malaria, since it could exist in the absence of the erythrocytic cycle.

The work of Fairley (1945, 1947) showed conclusively that in both vivax and falciparum malaria there was a cryptic phase in infections caused by mosquito bites during which pre-erythrocytic development must have been proceeding in some situation other than the blood stream. No other conclusion could be drawn from the results of his experiments, and he established the approximate duration of this cryptic phase as eight days in the case of vivax malaria and six days in the case of falciparum malaria.

As already stated above, the truth of this assumption had been established in the case of vivax malaria, but similar proof was still lacking in the case of falciparum malaria, and it was to obtain this that the experiment which is the subject of this paper was carried out.

Unlike the case in vivax malaria, we were unable to argue from monkey to man, since no suitable monkey parasite closely similar to *P. falciparum* was available for study, and a human experiment was necessary without any preliminary comparative work.

The fact that *P. falciparum* shows considerable differences in its morphology and life cycle from *P. vivax* at least raised the possibility that there might be certain differences in the nature of the cryptic stage as compared with those of *P. vivax* and *P. cynomolgi*, as well as in the site in the body where it occurred. It seemed probable, also, if we accept the persisting exo-erythrocytic cycle as the source of relapses in vivax malaria, that, although there would be a pre-erythrocytic cycle in falciparum malaria, it would be short-lived and end with establishment of the erythrocytic infection. This is a natural deduction from the well-known fact, so conclusively proved by Fairley's experiments alluded to above, that complete eradication of the erythrocytic infection in falciparum malaria ends the disease and relapses do not occur. This made the problem of choosing the correct day for the biopsy on the volunteer of great importance, as will be seen when the details of the experiment are described.

Description of Experiment

Anopheline mosquitoes of the species *A. maculipennis atroparvus* and *A. quadrimaculatus* were infected by feeding on two occasions on a subject carrying in his blood large numbers of gametocytes of a Rumanian strain of *P. falciparum*. These were maintained on blood meals for fourteen days, and mosquitoes were dissected and examined daily to follow the progress of the infection. These dissections revealed an infection rate of 93%. After ten days the dissections showed that the salivary glands had been invaded, and it was considered that four days afterwards would be the optimum time to feed them on the volunteer.

This operation was carried out on three days as follows, a total of 770 bites by the mosquitoes being recorded.

On October 10, 1949, 350 mosquitoes fed; on October 11, 95 fed, these being the mosquitoes that had failed to feed on the first day. On October 12, 325 fed: the majority of these were the mosquitoes of the first day of feeding having a second blood meal on the volunteer.

The dissections referred to had shown that 93% of the mosquitoes were infected and that the infections in the salivary glands were extremely heavy, so that the dosage of sporozoites injected by the 770 bites must have been enormous.

After a full consideration of all the factors involved, and influenced chiefly by the duration of the incubation period

in falciparum malaria after infection by mosquitoes as exemplified in Fairley's work already alluded to, we decided to perform a biopsy and remove a piece of liver from the volunteer by open operation 140 hours after the initial feed of the mosquitoes. This implied the assumption, later proved to be correct, that the exo-erythrocytic stages would be found in the liver as in the cases of *P. cynomolgi* and *P. vivax*. As the mosquitoes had fed on three successive days, it will be evident that the hypothetical pre-erythrocytic forms at the time of the biopsy would be at three different stages of development separated by approximately 24-hour intervals. At the operation, performed by Mr. C. Naunton Morgan, a wedge-shaped piece of liver measuring about 1 in. by $\frac{3}{4}$ in. by $\frac{1}{2}$ in. (2.5 by 1.9 by 1.25 cm.) was removed. This was divided into pieces of appropriate size and fixed in Carnoy's fluid. Sections cut from this material were stained by the colophonium-Giemsa method (Shortt and Cooper, 1948).

Microscopical Examination of the Sections

In the liver sections were found pre-erythrocytic schizogony forms of *P. falciparum* very similar to those of *P. cynomolgi* and *P. vivax* but with differences noted below.

As expected, three stages have been found. The most advanced forms probably correspond to those initiated on the first day of infection by the mosquitoes, and were approximately 140 hours old; the second correspond to the second day of infection, and were approximately 116 hours old; while the least-advanced correspond to the third day of infection, and were approximately 92 hours old.

It must not be supposed that the examples of these three stages were all sharply differentiated in their degree of development, since there were two infection periods, separated by several hours, on each of the days in which the infecting mosquitoes fed, and this was responsible for some grading in the degree of development of the schizonts without materially affecting the differentiation of the forms found in the three stages corresponding to the three "infection" days. All appeared to be contained in the parenchyma cells of the liver.

FIG. 2.—Five-day-old pre-erythrocytic stage of *P. falciparum* in human liver ($\times 700$).

Forms 140 Hours Old.—In these forms, which measured up to 60μ or more in length by about 30μ in width, the merozoites were already formed and the schizonts ready to rupture or already in process of rupturing and releasing the merozoites into the surrounding sinusoids. The cytoplasm of the schizonts stained a blue or blue-mauve colour and the nuclei or chromatin masses a reddish magenta. The shape of the schizonts varied from ovoid to lobose. In some cases, however, an apparent membrane was present the source of which seems obscure. The merozoites were very small, measuring about 0.7μ across, and extremely numerous.

Forms 116 Hours Old.—Until a very lengthy and detailed examination of our material has been made it will

FIG. 1.—Four-day-old pre-erythrocytic stage of *P. falciparum* in human liver ($\times 700$).

FIG. 3.—Six-day-old pre-erythrocytic stage of *P. falciparum* in human liver ($\times 700$).

not be possible accurately to date the forms younger than the fully developed schizonts ready to rupture. In all the younger forms the nuclei were larger but much less numerous and the cytoplasm more abundant, the staining reactions remaining the same. The schizonts, which were ovoid or bluntly lobose in shape, were not quite so large as in the previous stage, but a large example would measure about $50\ \mu$ by $36\ \mu$ in the long and short diameters. This would probably represent a late fifth-day form.

Forms 92 Hours Old.—The schizonts were considerably smaller than the previous form, measuring about $31\ \mu$ by $26\ \mu$ in their longer and shorter diameters. They tended to be ovoid in shape, but lobose forms or forms constricted in the middle were already appearing. The blue cytoplasm was coarsely granular and the nuclei were much less numerous than in the previous stage. As in the cases of *P. cynomolgi* and *P. vivax*, there was no cellular reaction to the presence of the parasitic bodies in the liver tissue in any of the three stages described.

Comment

In comparing the various stages described with those found in *P. cynomolgi* and *P. vivax* it will be evident that the pre-erythrocytic forms of all three species are closely similar. Points of difference are the larger size of the mature schizont of *P. falciparum*, the smaller size of the merozoites, and the much greater number of these in the schizonts. The full maturity of the schizonts in less than six days proves that schizogony in the pre-erythrocytic forms of *P. falciparum* proceeds much more rapidly than in either *P. cynomolgi* or *P. vivax*.

The general impression gained, however, is that the three species must be considered closely related and that the genus *Plasmodium*, as exemplified in these three species whose essentially complete life cycles are now known, is a compactly knit entity the members of which conform closely to the generic pattern.

Summary

For the first time the existence of a pre-erythrocytic stage in the life cycle of *P. falciparum* has been demonstrated and the forms at three stages of development are briefly described.

We express our great indebtedness to many helpers who have assisted at various stages in the carrying out of this successful experiment. Our special thanks are due to Mr. C. Naunton Morgan, who performed the open operation on the liver with remarkable skill and celerity, to Dr. C. Langton Hewer, who gave up valuable time to administer the anaesthetic to the volunteer, and to the staff of the Hospital for Tropical Diseases for their assistance and care throughout the experiment. Dr. W. D. Nicol, physician superintendent of Horton Hospital, has earned our gratitude by granting the most ample facilities for the initial infection of the mosquitoes at Epsom and their subsequent use on the human volunteer. Mr. W. Cooper, chief technician, Miss Maryon, and Mrs. P. Lee gave invaluable and skilled assistance and spared neither time nor trouble in many tedious technical operations. The one indispensable actor in the piece was the human volunteer, Mr. C. H. Howard, who not only cheerfully, but on his own initiative, offered himself for this experiment and thereby helped greatly to advance scientific knowledge on an important aspect of malariology.

REFERENCES

- Fairley, N. H. (1945). *Trans. R. Soc. trop. Med. Hyg.*, **38**, 311.
 — (1947). *Ibid.*, **40**, 621.
 Shortt, H. E., and Cooper, W. (1948). *Ibid.*, **41**, 427.
 — and Garnham, P. C. C. (1948). *Nature*, **161**, 126.
 — Covell, G., and Shute, P. G. (1948). *British Medical Journal*, **1**, 547.

REHABILITATION AND RESETTLEMENT OF THE TUBERCULOUS*

BY

FREDERICK HEAF, M.A., M.D., F.R.C.P.

Adviser in Tuberculosis to the Ministry of Health; Senior Medical Officer, London County Council

In all matters concerning the resettlement and rehabilitation of the tuberculous three factors are of paramount importance. The first is that it is impossible to be sure that a tuberculous person has completely recovered from his disability; the second is that there are no means of assessing a tuberculous person's capacity for work except by trial and error; and the third is that when the disease is active it is usually infectious. These three factors differentiate the problem of other disabled persons from that of the tuberculous and make it necessary to have special schemes to meet the requirements of the latter group. I propose to examine in more detail the three factors with special reference to resettlement and rehabilitation.

1. Uncertainty about Recovery

It is not easy to find reliable statistics on the relapse rate of those who have been treated in sanatoria. There are many reports on the post-sanatorium mortality rate which are helpful, but these do not indicate the amount of chronic invalidism amongst the tuberculous population. Here are some conclusions taken from studies of after-histories of patients.

Bashford and Scott (1936) in their report on the health of 3,755 Post Office workers state: "Of those who incur pulmonary tuberculosis not more than 50% are able to return to work even after prolonged periods of treatment, and of those who return there has been a further wastage of about 48% as a result of recurrent pulmonary tuberculosis, or other form of ill-health, by the end of ten years, the heaviest wastage having occurred in the first six years."

Tattersall (1949), in a five-year follow-up of sputum-positive cases, found that 10% of the "recovered" cases deteriorated after an average period of six years as "recovered." He suggests that a patient whose working capacity is reduced by phthisis is not always a short-term social liability, and that it is doubtful if there is any justification in discharging from supervision any tuberculous person who has had a positive sputum.

Such information, although emphasizing the need for special measures for rehabilitating the tuberculous, is not particularly encouraging, and it would be as well at this point to inquire why the tuberculous relapse and die. A satisfactory answer to this query cannot be given; all that can be said is that certain conditions predispose to activity of the disease. Which of these conditions is the most important varies from individual to individual. Under-nutrition, overstrain, anxiety, acute respiratory infections, insufficient rest, and certain occupational conditions are factors that influence the state of the lesion, and the facts may be summarized in a brief but vague way by saying that anything that lowers the resistance of the body to infection will increase the possibility of a breakdown from tuberculosis. This is really begging the question, as resistance is a term which cannot be defined. The inability of the body to rid itself of the tubercle bacillus is well known, and he would be a bold man who said that in any arrested

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