9,000 units. The treated mice died in the same interval as the infected, untreated controls.

(3) Relapsing fever (*"S. novyi"* strain). Eleven mice were inoculated intraperitoneally with 0.25 cc heparinated pooled blood from five mice showing heavy infections with *S. novyi.* Twenty-four hours later the infections were moderately heavy and treatment of six of the mice was started with penicillin sodium. The remaining 5 mice served as controls. The treated mice received intraperitoneally 1,000 units in 1 cc saline for a first dose. Every 3 hours thereafter, for 48 hours, each animal received an additional 500 units of the drug. The total dose for each treated mouse was 9,000 units. The first effects of the drug were observed 6 hours after the first dose. At this time the infections had decreased in intensity to about one fortieth in the treated mice, whereas they increased about 50 per cent. in the untreated animals. At the end of 27 hours no spirochaetes were microscopically visible in the treated mice, whereas in the untreated animals they averaged 140 in a single oil immersion field. Sixty hours after treatment was started, 2 of the apparently cured mice were sacrificed. The citrated heart blood of each mouse was inoculated intraperitoneally into two new mice. No infections resulted.

In a second experiment a relapsing fever mouse, sacrificed after receiving 4,000 units in 19 hours, was found to be a carrier, although no spirochaetes were found in a thick drop of its blood.

From the results of these preliminary experiments, it is evident that penicillin sodium, in the very large doses employed, was inactive against *Trypanosoma lewisi* and *Toxoplasma,* but was spectacularly effective in the treatment of relapsing fever.

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HEPATIC DYSFUNCTION IN MALARIA

EVERY student of the subject is aware that enlargement and tenderness of the liver and even jaundice may occur in the various forms of malaria. Nevertheless, relatively little attention has been given to the possibility that hepatic dysfunction and its associated derangements in metabolism can exist in this disease. Slatineau and Sibi,[1] and Phocas[2] did note that some degree of transitory hepatic insufficiency exists in nearly all cases, but their data has not received general acceptance. More recently Kopp and Solomon[3] studied the influence of induced tertian malaria on the liver function of nine patients under treatment for general paresis. They noted a transient disturbance in liver function which usually cleared up within three to six weeks after the termination of the malaria.

With the increasing incidence of malaria consequent to the war, it became important to establish further the probability that an associated liver dysfunction is more frequent than is generally acknowledged. Towards this end, the status of the function of the liver was studied in a series of malaria patients by means of various tests and constituents of the blood. The present preliminary report deals with two cases without a previous history of malaria before the present attack, six with recurrent malaria and a history dating back as long as six months to two years, and two patients with a definite history but no evidence of malaria at the time of study.

The preliminary pertinent data obtained at various intervals during the patients' hospitalization are summarized in Table 1 and reveal that every patient with malaria had an abnormal cephalin cholesterol flocculation test. The majority of the patients also demonstrated an increased sedimentation rate, an anemia, a high serum globulin and, in fewer instances, a slightly increased icteric index. For purposes of comparison another flocculation test, the Kahn test for syphilis, was performed at the same time as the cephalin cholesterol flocculation test. In every instance a negative result was obtained.

Many studies with the cephalin cholesterol test have established that this procedure is an excellent, sensitive measure of hepatic damage. The data summarized previously, therefore, may be interpreted as indicating that in nearly every case of malaria some degree of liver damage may exist. Since the initial test was performed in several instances before any therapy was instituted, it is probable that neither atabrine nor quinine are factors in the production of the hepatic damage.

The presence of liver damage in malaria necessitates revision of modern treatment, since not only is it necessary to administer the specific drugs aimed at the elimination of the plasmodia, but it is necessary also to institute measures which may result in a restitution of the liver to normal. Such measures are the administration of high carbohydrate, high protein, high vitamin diets and not the administration of "only fluids during the course of the fever," as is advocated by some of the leading students of malaria.

The efficacy of a diet aimed at improving the status of the liver is suggested by the fact that patients thus treated may show a rapid disappearance of the positive cephalin cholesterol flocculation test, while patients not treated in this manner show a positive floc-

[1] A. Slatineau and M. Sibi, *Arch. Roumanes de Path. Exper. et Microbiol.,* 7: 529, 1934.

[2] E. Phocas, *Rev. Med. et Hygiene Tropicales,* 29: 246, 1937.

[3] I. Kopp and H. C. Solomon, *Am. Jour. Med. Sci.,* 205: 90, 1943.

TABLE 1
HEPATIC FUNCTION IN MALARIA

Name	Plasmodium	History	R B C	Reticulocytes	Sedimentation rate	Total cholesterol	Blood sugar	Icterus index	Serum albumin	Serum globulin	Kahn	Bromsulfalein	Cephalin-cholesterol
			M/cmm	/100	mm/hr	mgm/100	mgm/100		gm/100	gm/100		%/45 min	
PL	Falciparum	Acute	2.66	..	8.1	...	...	18	5.5	1.5	Neg.	..	+++
JA	0	Chronic (?)	3.70	..	31.5	195	69	6	..	..	Neg.	0	++
HB	Vivax	Acute	3.80	0.5	51	110	111	11	4.0	3.0	Neg.	0	++++
HL	0	Chronic (?)	4.30	0.1	14	280	88	5	3.7	2.5	Neg.	..	++
OW	Vivax	Recurrent	4.11	..	...	230	79	18	4.0	2.0	Neg.	..	++
CH	Falciparum	"	4.33	0.5	...	225	...	10	4.5	2.2	Neg.	..	+++
AMcC	Vivax	"	3.77	1.4	...	155	...	11	4.5	2.9	Neg.	..	++++
IVS	0	"	4.17	0.4	29	205	91	5	4.9	3.1	Neg.	0	++
CRR	Vivax	"	4.4	1.9	30	120	64	4	4.0	3.3	Neg.	12	++++
GER	Vivax	"	4.7	0.9	10	190	101	9	4.3	2.9	Neg.	0	+++

culation reaction as long as one year after the last attack.

Further and more extensive studies as to the status of the liver in malaria are imperative. It may be important also, to determine the relationship of liver damage to the incidence of recurrences and the development of immunity. Such studies will form the basis of a more complete report.

CONCLUSIONS

(1) The cephalin cholesterol flocculation test was positive in all ten cases of malaria of varying duration. This is interpreted as indicative of the presence of hepatic damage.

(2) It is proposed that in addition to the specific drugs, a high carbohydrate, high protein and high vitamin diet be administered early in the therapeutic régime.

Major Mirsky is indebted to Dr. David Klein, The Wilson Company, Chicago, Ill., for generous supplies of the cephalin-cholesterol mixture.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PENICILLIN ASSAY

Outline of Four-Hour Turbidimetric Method

THE following method of determining the potency of penicillin solutions is advantageous in that it is conveniently set up and makes possible the turbidimetric reading of the test in the same test-tubes in which the culture is grown. In these respects it is believed to be more practical than turbidimetric methods proposed by others.[1]

Procedure: Into a duplicate series of nine sterile, plugged and standardized test-tubes (18 × 140 mm) place aseptically, in order, the amounts of sterile Veal-Glucose Broth[2] noted in column 4 of Table 1. These

TABLE 1

Units of penicillin (standard)	Penicillin Sol. added: Strength	Penicillin Sol. added: cc	Amount of broth in tubes	Optical density: Standard No. 1	Optical density: Standard No. 2	Optical density: Unknown
None	..	..	9.6	.33	.32	.30
0.05	0.5 per cc	0.1	9.5	.30	.29	.29
0.10	"	0.2	9.4	.27	.26	.26
0.15	"	0.3	9.3	.22	.22	.23
0.20	"	0.4	9.2	.19	.20	.21
0.25	"	0.5	9.1	.17	.18	.19
0.30	"	0.6	9.0	.15	.15	.17
0.35	"	0.7	8.9	.15	.15	.16
0.35	"	0.7	9.3	.00	.00	.00

[1] J. W. Foster, *Jour. Biol. Chem.*, 144: 285, 1942; J. W. Foster and H. B. Woodruff, *Jour. Bact.*, 46: 196, 1943; J. W. Foster and B. L. Wilker, *Jour. Bact.*, 46: 387, 1943.

[2] Veal-Glucose Broth: 1. To 500 gm ground veal from suckling calves 6–8 weeks old, which have not been slaughtered more than one week, add 1,000 cc of distilled water and soak overnight, in refrigerator. 2. Boil 15 minutes, strain through cheesecloth and make up to original volume of water. 3. Sterilize at 15 pounds pressure for 45 minutes. For convenience, a quantity of this base-infusion can be made at one time and stored. 4. Filter sterile veal infusion prepared as above through wet, coarse filter paper. 5. Add Bacteriological Peptone (P.D. & Co.), 1 per cent.; Sodium Chloride (Diamond Crystal), 0.5 per cent.; Dextrose C.P., 0.1 per cent. 6. Heat to dissolve. 7. Adjust to final pH of 8.0 (8.6 at this point). 8. Heat at flowing steam for 15 minutes. 9. Filter through wet fine paper. 10. Fill as desired. 11. Sterilize at 15 pounds pressure for 20 minutes.