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.

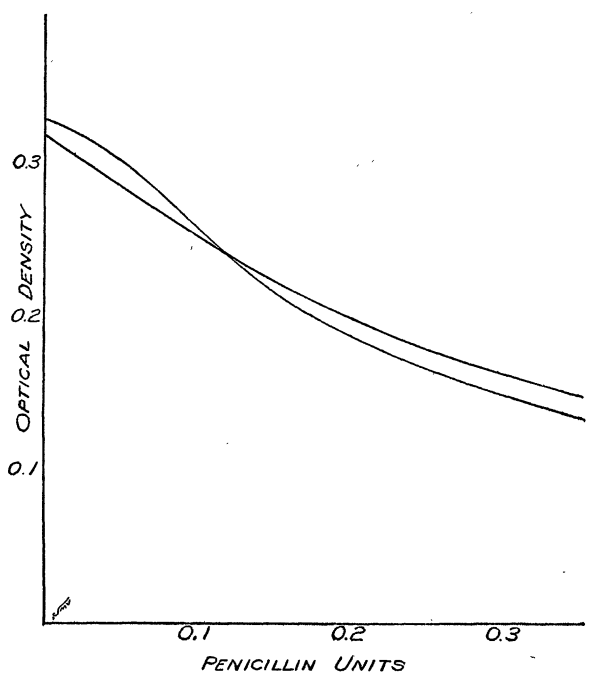
are for the standard which is run in duplicate. For each unknown solution to be assayed, prepare one additional row of nine tubes.

Prepare a solution containing 0.5 unit per cc of the penicillin standard in cold sterile distilled water and add to the broth tubes according to the amounts in column 3. From estimated values of units for the unknown samples, prepare a solution of each which contains an estimated 0.5 unit per cc. Add these solutions to their respective rows of tubes in the same manner as the standard. Place into each tube (except for the last which serves as a colorimetric and sterility control) 0.4 cc of an 18–20 hour culture of *Staphylococcus aureus* (National Institute of Health No. 209). The culture is prepared by inoculating a flask containing Veal-Glucose Broth from an agar slant. This resulting suspension, which is used as the inoculum, should have an optical density of about 0.4.

Place the tubes into an incubator or constant temperature water-bath at 37° C for 4 hours or until the optical density of the control tube reaches about 0.30 (0.27 to 0.34 has been found to be satisfactory). (A constant temperature water-bath has been found to give more consistent results and hence a smoother curve). At the end of the growing period immerse the tubes in cold water (about 10° C or less) to stop active growth of bacteria. After cooling, the tubes should be wiped dry and shaken thoroughly.

Measure the density of each tube by means of a photoelectric colorimeter[3] using a red filter. These values are recorded opposite their corresponding tubes, as may be seen in Table 1, a typical protocol.

The densities are plotted as the ordinates against penicillin units as the abscissae. A smooth curve is drawn through these points. This is done for each row of tubes representing the standard, thus giving two curves which correspond to the duplicate standard. A line is then drawn halfway between these two curves and serves as the average curve which is employed in the calculation of the number of units in the samples of unknown strengths. This calculation is explained in Table 2.




[3] Lumetron. Manufactured by Photovolt Corp., New York City.

TABLE 2

| A Units of penicillin in standard | Optical density of unknown | B Units of unknown (read from curve) | C Estimated units of unknown | Units of unknown $\frac{B}{A}$ C = |
|---|---|---|---|---|
| 0.05 | 0.29 | 0.056 | 5000 | 5600* |
| 0.10 | 0.26 | 0.096 | " | 4800 |
| 0.15 | 0.23 | 0.136 | " | 4500 |
| 0.20 | 0.21 | 0.168 | " | 4200 |
| 0.25 | 0.19 | 0.210 | " | 4200 |
| 0.30 | 0.17 | 0.260 | " | 4330 |
| 0.35 | 0.16 | 0.290 | " | 4150 |
| | | | | Av. = 4370 |

* Not used in calculation because 5600 is definitely out of range.

Values obtained by this method are characteristically in good agreement. Consecutive assays on two samples were 48300, 47600, 45300, 49400 and 1675, 1516, 1300, 1580, respectively. In five consecutive days the units per cc of one standard as calculated from the curve of its duplicate were 0.495; 0.52; 0.45; 0.52 and 0.49, respectively.

Appreciation is hereby expressed for the technical assistance given by Dr. J. M. Vandenbelt of this laboratory.

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