to a series of injections of type rh blood in an attempt to induce sensitization to the factor hr′ or Hr_0, or both. Nineteen of these individuals received 2 injections; of these 13 received a third injection, 8 received a fourth injection, 5 received a fifth injection, while 4 received a sixth injection. Not one of the individuals of this series showed any Hr antibodies at any time. This, despite the smaller number of individuals injected, is in sharp contrast to our experiences with sensitization against Rh_0 factor. Moreover, these results conform with the conclusions based on clinical observations that the Hr factors are much weaker antigens than the Rh factors.[7]

Summary. The results of experiments on sensitization of male type rh individuals by intravenous injections of Rh_0-positive blood at intervals of 3 to 4 months are described. Of 47 subjects receiving 2 injections, 38.3% became sensitized; after 3 injections, the incidence of sensitization rose to 54.4%; after 4 injections, the percentage rose to 63.5%, while after 5 injections the incidence of sensitization was 67.2%. Because of the small number of individuals receiving more than 5 injections, it is not possible to state with certainty whether the incidence of sensitization approaches a maximum limit somewhere between 70 and 80% of individuals injected, or whether all individuals eventually would become sensitized providing they are given a sufficient number of injections. In any event, it is obvious that the deliberate injection of Rh-positive blood is a potent means of sensitizing Rh-negative individuals.

In contrast to these results, not one among 19 type Rh_1Rh_1 individuals receiving repeated injections of type rh blood became sensitized to the Hr factors. These results confirm the conclusion based on clinical observations that the Hr factors are much weaker antigens than the Rh factors.

The author wishes to express his appreciation to Miss Helen Reiss, who assisted with the blood injections, and to Mrs. E. B. Gordon, Mrs. L. Katz, and Mrs. C. Mazzarino for their assistance in carrying out the Rh antibody tests. He is also indebted to Mr. I. H. Gilbert of the Certified Blood Donor Service for his cooperation in providing the blood donors who submitted to the blood injections.

[7] Wiener, A. S., *J. Lab. and Clin. Med.*, 1948, **33**, 985.

17000

Glycolysis in *Plasmodium gallinaceum.**

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For many years a practical method of cultivating the malaria plasmodia has been sought, and recently one has been devised by Ball and his colleagues[1,2] for the erythrocytic stages of *Plasmodium knowlesi*, a parasite of monkeys. Monkeys, however, are expensive and much less easily maintained in the laboratory than birds, and for this reason it is important that a good culture technic be devised for the avian plasmodia,‡ which are equally suited for the screening of antimalarial drugs and for other types of research in malaria. It

* Aided by a grant from the National Institute of Health.

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[1] Ball, E. G., Anfinsen, C. B., Geiman, Q. M., McKee, R. W., and Ormsbee, R. A., *Science*, 1945, **101**, 542.

[2] Geiman, Q. M., Anfinsen, C. B., McKee, R. W., Ormsbee, R. A., and Ball, E. G., *J. Exp. Med.*, 1946, **84**, 583.

‡ Hawking[3] has devised a tissue culture technic for growing the exoerythrocytic stages of several species of avian plasmodia.

[3] Hawking, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1945, **39**, 245.

is also clear that an integrated picture of the malaria problem cannot be had until our knowledge includes as much about the non-human species of malaria as it does of the plasmodia of man.

For these reasons, the authors attempted to adapt the Ball culture method to *Plasmodium gallinaceum*, the parasite of chicken malaria, but with only partial success, and it then seemed evident that some careful studies of its biochemistry would be necessary before greater success could be hoped for. Since the addition of more glucose to the medium than originally called for seemed to improve its performance somewhat, a detailed and quantitative study of glycolysis in this species was undertaken.¶

Materials and Methods. The strain of *Plasmodium gallinaceum* used was obtained from Dr. Gilbert Otto of the Johns Hopkins University School of Hygiene and Public Health. Inoculation of the chicks was done either intraperitoneally or intramuscularly with brain suspensions or parasitized blood, usually when they were about three weeks old. Blood for experimental use was obtained by intracardial puncture, without anesthesia, to insure normal blood sugar levels. To avoid possible effects of a developing immunity, chicks were used during the period of rising parasitemia. The anticoagulant was Lederle heparin.

After removal from the bird the blood was taken immediately to the constant temperature (40°C) room, 0.5 ml was saved for immediate examination, and the balance placed in a rocker dilution tube, equilibrated with 5% CO_2 and 95% air, and rocked according to the Ball cultivation technic, samples being taken at regular intervals for glucose determinations, which were always made in duplicate. These were plotted against time and the hourly rate of glucose consumption calculated. This last was done per ml of blood, both normal and parasitized, and per mature red cell, reticulocyte, and parasite.

Glucose levels were determined by the Folin-Malmros method (Klett-Summerson Clinical Manual), except that a ferric duponal solution[4] was substituted for the ferric gum-ghatti solution originally specified, thus avoiding the partial precipitation of the Prussian blue which sometimes occurred with the gum ghatti. The glucose values usually differed from their mean by no more than ± 3 mg %. Erythrocyte and parasite counts were made on each sample, enough parasites being counted to keep the error within 10%[5] Differentials were also made. The stain used was the J.S.B.[6]

Whole undiluted blood was employed for all determinations. For the study of the glucose consumption of reticulocytes, chicks were made anemic by 3 previous daily injections of phenylhydrazine hydrochloride.

Results. The hourly glucose consumption rates of normal chicken blood and of blood containing a high proportion of reticulocytes (79.5%) are illustrated in Fig. 1 and 2 respectively. Fig. 3 shows in similar fashion the hourly glucose consumption of parasitized blood. With larger numbers of parasites, or greater proportions of reticulocytes, curves with even steeper initial slopes would be obtained.

Five determinations were made of the hourly glucose consumption of unparasitized

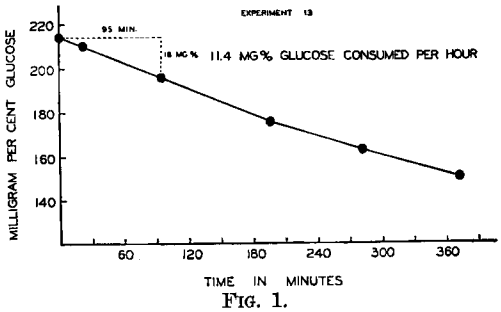

FIG. 1.

Glucose consumption of unparasitized blood containing approximately 100% adult red cells.

¶ Since the writing of this report, the paper by Marshall (*Brit. J. Pharm.*, **3**, 1) has come to our attention. Although his determinations of oxygen uptake of parasitized blood indicate glycolysis values for *P. gallinaceum* essentially like ours, added glucose failed to stimulate respiratory activity.

4 Klenshoj, N. C., and Hubbard, R. S., *J. Lab. and Clin. Med.*, 1939, **25**, 1102.

5 Hartman, E., *Am. J. Hyg.*, 1927, **7**, 407.

6 Singh, J., and Bhattacharji, L. M., *Ind. Med. Gaz.*, 1944, **79**, 102.

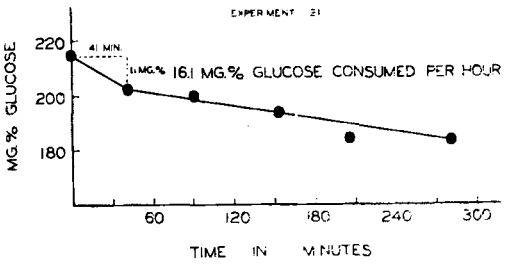

FIG. 2.

Unparasitized blood containing approximately 20.5% adult red cells and 79.5 reticulocytes.

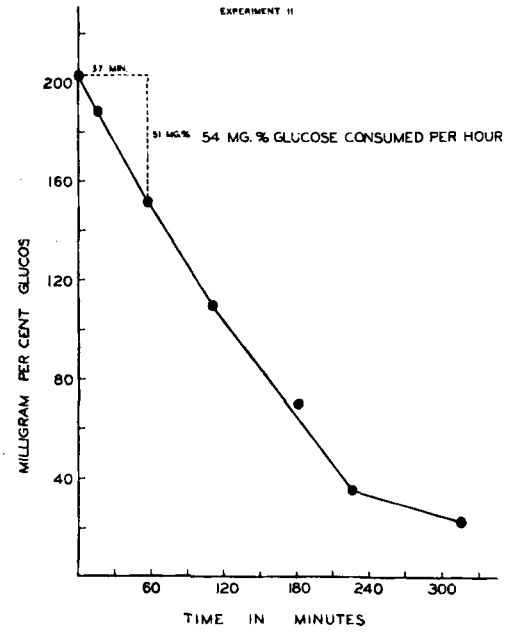

FIG. 3.

Glucose consumption of parasitized blood, containing 89.5% adult red cells, 10.5% reticulocytes, and 178 parasites per 100 red cells (Type A, 59%, Type B 36%, and Type C 5%).

blood, 3 of these on normal and 2 on highly anemic samples. The results were 0.114, 0.064, and 0.083 mg per ml on the former, and 0.161 and 0.360 mg per ml on the latter. From these figures the hourly glucose consumption per 10^9 red cells (for both mature erythrocytes and reticulocytes) was calculated.‖ The mean value for mature erythrocytes was found to be 4.34×10^{-2} mg and for reticulocytes 3.06×10^{-1}. Thus the average mature red cell has a metabolic rate only about one-seventh that of a reticulocyte.

The hourly glucose consumption rate of parasitized cells is much higher, although it depends on the size and stage in the asexual cycle of the parasite. Five determinations were made of the glucose consumption of parasitized blood. (Table I) The mean value, in mg per hour per ml, was 0.48—a figure substantially higher than any of the rates obtained for unparasitized blood, normal or anemic.

To make such values really comparable with those obtained for unparasitized blood it is necessary to know both the parasite count, and the proportions present of parasites of different sizes. Silverman and his colleagues[7] distinguished 3 types. Merozoites freshly formed by segmentation they called "Type A." Somewhat larger stages, having up to 4 masses of chromatin, they designated as "Type B," and all others were called "Type C." They found the surface areas of these 3 types to be approximately 1, 12, and 48 square micra.

Using this scheme of classification, we determined their hourly glucose consumption rates to be about 3.00×10^{-11} mg, 3.60×10^{-10} mg, and 1.44×10^{-9} mg respectively. An "average" parasite (one having the mean of these 3 areas) consumed glucose about 14 times as fast as an adult erythrocyte and twice as fast as a reticulocyte. The data on which these and other calculations were based is summarized in Table I.

From these data the glucose consumption rates per square micron of surface area may also be calculated, both for erythrocytes and parasites. We found this to be 3.42×10^{-13}

‖ This involved the use of the formula

$$\text{mg glucose per hour per ml} = E(AX \times RX')$$

in which

$$A = \frac{\text{adult erythrocytes}}{\text{total erythrocytes}}$$

$$E = \frac{\text{erythrocytes per mm}^3}{10^6}$$

$$R = \frac{\text{reticulocytes}}{\text{total erythrocytes}}$$

X = hourly glucose consumption (mg) of 10^9 red cells

X' = hourly glucose consumption (mg) of 10^9 reticulocytes

[7] Silverman, M., Ceithaml, J., Taliaferro, L. G., and Evans, E. A., *J. Inf. Dis.*, 1944, **75**, 212.

TABLE I.
Summary of Experimental Data.*

A. Normal Unparasitized Blood.

Experiment	E	A	R	Mg glucose per hr per ml blood	X
13 (Fig. 1)	1.91	1.000	0.000	0.114	5.97×10^{-2}
14	1.86	0.995	0.005	0.064	3.36×10^{-2}
15	2.25	1.000	0.000	0.083	3.68×10^{-2}
				Mean:	4.34×10^{-2}

B. Anemic Unparasitized Blood.

Experiment	E	A	R	Mg glucose per hr per ml blood	X'
21 (Fig. 2)	0.67	0.205	0.795	0.161	2.91×10^{-1}
22	1.58	0.335	0.665	0.360	3.21×10^{-1}
				Mean:	3.06×10^{-1}

C. Parasitized Blood.

Experiment	E	A	R	P	a	b	c	Mg glucose per hr per ml blood	Mg glucose per hr per 10^7 μ^2 par. area
11 (Fig. 3)	1.33	0.895	0.105	178	59	36	5	0.54	2.57×10^{-4}
12	1.81	0.990	0.010	61	15	76	9	0.42	2.27×10^{-4}
16	0.78	0.650	0.350	200	4	93	3	0.75	3.24×10^{-4}
18	1.87	0.810	0.190	10.5	18.5	16.7	64.8	0.35	2.66×10^{-4}
19	1.34	0.562	0.438	19.8	0.0	98	2	0.36	4.27×10^{-4}
								Mean:	3.00×10^{-4}

* Abbreviations used in Table I, and in the formulae:
a, b, c = percentage distribution of 3 parasite types

$$E = \frac{\text{erythrocytes per mm}^3}{10^6}$$

$$A = \frac{\text{adult erythrocytes}}{\text{total red cell count}}$$

$$R = \frac{\text{reticulocytes}}{\text{total red count}}$$

P = parasites per 100 red cells

X = mg glucose per hr per 10^9 red cells

X' = mg glucose per hr per 10^9 reticulocytes

TABLE II.
Glucose Consumption (mg per hour).

Authority	Erythrocytes (per red cell)	Erythrocytes (per μ^2 of area)	Parasites (per μ^2 area)	Ratio (col. 2/col. 3)
Our work	4.34×10^{-11}	3.42×10^{-13}	300×10^{-13}	1/87.8
Silverman *et al.*	6.25×10^{-12}	4.92×10^{-14}	390×10^{-14}	1/79.0
Ratio (our values to Silverman's)	6.95/1		7.7/1	

mg per hour for normal erythrocytes (adult), and 300×10^{-13} for parasites. Both values greatly exceed those of Silverman and colleagues (Table II), and we believe the differences are probably due to the different treatment given the blood in our work, since we used no diluting agent and did not wash the cells before making the tests, thus keeping the cell environment more nearly physiologically normal.

To make these calculations, and also to predict the glucose consumption of cultures containing any given number of parasites, the following formula was developed:

$$W_t = 4.34E \times 10^{-2} (A + 7.05R + 6.92P \times 10^{-5} (a + 12b + 48c))$$

in which

W_t = mg glucose consumed per hour per ml of parasitized blood
a, b, and c = percentage distribution of 3 parasite types
A, E, R, X, and X' are as indicated above (formula used in computing glucose consumption of unparasitized blood)

The formula is easily derived, assuming

that the glucose consumed by parasitized blood (W_t) is the sum of that utilized by adult red cells (W_A), reticulocytes (W_R), and parasites (W_P) in unit time.

W_A = number of red cells in 1 ml blood × hourly glucose consumption of 1 red cell
or $(10^9 \times A \times E) \times (4.34 \times 10^{-11}) = 4.34AE \times 10^{-2}$ mg

W_R = number of reticulocytes in 1 ml blood × hourly glucose consumption of 1 reticulocyte
or $(10^9 \times R \times E) \times (3.06 \times 10^{-10}) = 3.06RE \times 10^{-1}$ mg

W_P = total square micra of parasite surface area × hourly glucose consumption per square micron
or $(PE \times 10^5 (a + 12b + 48c)) \times (3.00 \times 10^{-11}) = 3.00PE \times 10^{-6} (a + 12b + 48c)$ mg

Therefore

$$W_t = (4.34AE \times 10^{-2}) + (3.06RE \times 10^{-1}) + (3PE \times 10^{-6}) (a + 12b + 48c) = 4.34E \times 10^{-2} (A + 7.05R + 6.92P \times 10^{-5} (a + 12b + 48c))$$

Discussion. In making the calculations and constructing the formula above it has been assumed that (1) the blood cells are in an essentially normal environment for at least the first 30 minutes, and therefore metabolize normally, (2) the metabolism of leucocytes and thrombocytes is normal and approximately constant in each sample, (3) the levels of any hormones influencing red cell metabolism are constant, or negligible in effect, (4) the metabolism of the infected red cell is undisturbed by the presence of the parasite, and (5) the metabolism of the gametocytes is the same, or not greatly different from that of asexual forms of like size. The fourth and fifth assumptions may be open to question, but it has so far not been possible to put them to experimental test.

Despite certain sources of error inherent in the experimental procedures, such as the counting technics, agreement between the experimentally determined and the values predicted by the formula has been close, the mean deviation between the two having been only ± .036 mg glucose per ml per hour.

Summary and Conclusions. A study of the glucose requirements of *Plasmodium gallinaceum* has been made, and formulae derived for predicting the hourly glucose consumption rates of parasitized and unparasitized blood. These should be useful in predicting the amounts of glucose required for cultures and indicative of the *in vivo* drain on carbohydrate stores. Normal chicken blood was found to have an hourly glucose consumption rate of approximately 8.7 mg per 100 ml, and values as high as 74.7 mg per 100 ml were observed for parasitized blood. The glucose consumption per square micron per hour was 3.42×10^{-13} mg, 2.41×10^{-12} mg, and 3.00×10^{-11} mg for adult red cells, reticulocytes, and parasites respectively.

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Riboflavin and Growth of Tubercle Bacilli.

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It was reported previously (Hou;[1] Farber and Miller[2]) that riboflavin deficiency was common among patients suffering from tuberculosis and that phlyctenular conjunctivitis or keratitis frequently observed in tuberculous patients responded to riboflavin therapy (Hou[3]). It was postulated (Hou[1]) that although tubercle bacilli will grow in the ab-

* On leave from The Institute of Nutrition, China, and on a traveling fellowship of the World Health Organization of the United Nations.

1 Hou, H. C., *Chinese J. Med.*, 1943, **62**, 181.

2 Farber, J. E., and Miller, D. K., *Am. Rev. Tuberc.*, 1943, **48**, 412.

3 Hou, H. C., *Chinese Med. J.*, 1940, **58**, 616.