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.

17001

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-

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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.

TABLE I.
Influence of Riboflavin on the Growth of Tubercle Bacillus.

Medium, character of	% transmission with air blank as reference			
	Control, no riboflavin	Riboflavin, % conc.		
		0.25	0.5	1.0
Vit. free	68.5	66.4	67.1	66.3
+ Vit. A	61.8	61.1	—	—
+ Vit. A + Biotin	58.4	61.4	—	—
+ Vit. A + Thiamine	58.8	58.6	—	—
+ Vit. A + Biotin + Thiamine	59.3	57.2	—	—

sence of riboflavin (Boissevain *et al.*[4]; Street and Reeves[5]) they may remove riboflavin from the host during the multiplication and thus bring about a deficiency condition. The present experiment was carried out to determine first whether the addition of riboflavin to the medium would promote the growth of tubercle bacilli and second whether there would be a loss of riboflavin from the medium during the growth of the organism.

Procedure. Human tubercle bacilli of a slightly virulent strain TBI† were used. The culture medium was made according to the formula of Dubos and Middlebrook[6] with the following modifications: Twenty ml of hydrolyzed vitamin free casein solution (1 ml corresponds to 100 mg casein) were used instead of 2 g enzymatic digest of casein. Tween 80 was purified according to the method of Davis[7] while bovine albumin was omitted.

For the study of the effect of riboflavin on the growth of tubercle bacillus 10 ml portions of the medium were distributed in 50 cc Erlenmeyer flasks. For each series 4 to 6 flasks were used as controls while a similar number was used for each of 3 different levels of riboflavin, namely, 0.025 mg, 0.05 mg, and 0.1 mg per 10 ml. After the addition of riboflavin or other vitamin solutions, the final volume of liquid in each flask was made up to the same amount by the addition of sterile distilled water. To prevent the destruction of riboflavin by light the flasks containing the riboflavin solution were covered with black cloth or paper. All flasks containing the medium were incubated for 24 hours to see if any was contaminated. One-tenth ml of a liquid tubercle bacillus culture 7 to 10 days old was added to each flask. The amount of bacterial inoculum was estimated to be 0.0014 mg, 0.014 mg and 0.14 mg for different series of experiments. After 4 to 7 days incubation at 37°C the degree of growth was determined turbidimetrically by means of a Coleman Junior Spectrophotometer. The metal screw-capped test tubes used for the reading were read first with air as a reference. The wave length was set at 560 mμ.

For the study on the loss of riboflavin from the medium, 4.0 mg of riboflavin in 80 ml of 0.02 N acetic acid solution were added to 1 liter of medium so that each ml of the medium contained about 4 γ of riboflavin. One hundred ml were distributed in each of 10 flasks, which were protected from light by wrapping with black cloth or paper. After incubation for 24 hours, to determine if the medium re-

[4] Boissevain, C. H., Drea, W. F., and Schultz, H. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 481.

[5] Street, H. R., and Reeves, R. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 641.

† Originally came from the Bureau of Animal Industry.

[6] Dubos, R. J., and Middlebrook, G., *Am. Rev. Tuberculosis*, 1947, **54**, 334.

[7] Davis, B. D., *Arch. Biochem.*, 1947, **15**, 359.

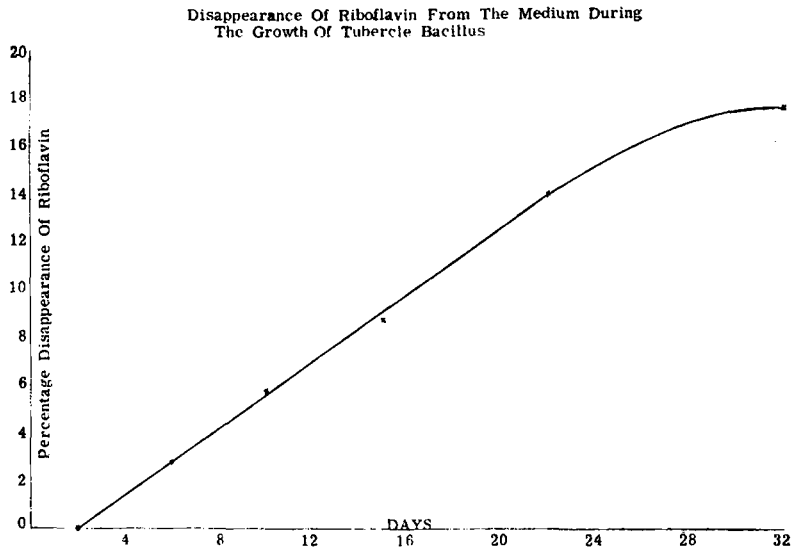

FIG. 1.

mained sterile, one-half the number of flasks were kept as controls while the remaining were inoculated with tubercle bacillus. All flasks were incubated at 37°C. At intervals of 3 days to one week 5 ml samples were removed from each flask and autoclaved at 15 lb for 10 minutes. Smears were made at each sampling to exclude any contaminated organism. If present the sample was discarded. The samples were subsequently centrifuged at high speed for 10 minutes to remove all solid particles and/or bacteria. The supernatant fluid was used for the analysis of riboflavin content, which was determined microbiologically according to a modified method of Snell and Strong[8] and fluorometrically by a composite procedure of several methods.[8]

Results and Discussion. Effect of riboflavin on growth of tubercle bacilli. The results of the study are summarized in Table I. Each figure represents the average of 10 to 20 different sets of readings. With the medium free from riboflavin the growth of tubercle bacilli was similar to that in media containing different levels of riboflavin, namely, 0.25, 0.5 and 1.0%. The addition of vitamin A, thiamine and biotin, individually or together, to the medium with or without riboflavin also did not influence the growth of the organism.

Effect of growth of tubercle bacillus on the level of riboflavin in the medium. The results of this study are shown in Fig. 1. During the first 22 days of incubation the percentage of disappearance of riboflavin from the medium plotted against time in days gave a straight line. After that time the curve began to flatten off. The disappearance of riboflavin apparently was proportional to the growth of the organisms. By the fourth week the growth had reached its maximum and the disappearance of riboflavin became correspondingly slower. On the whole the loss of riboflavin, however, was not as great as one would expect with the abundance of growth. It is conceivable that some dead organisms might have contributed some riboflavin to the medium. Foster[9] recently reported that riboflavin could be converted into lumichrome by an organism which he named *Pseudomonas riboflavinus nov. sp.* The lumichrome crystals were needle-like in appearance and could be isolated and identified by a characteristic absorption spectrum. In the present experiment similar crystals were first observed in stained smears and then in unstained liquid smears. Using Foster's technic

[8] Association of Vitamin Chemists, Inc., Interscience Publishers, Inc., New York, 1947.

[9] Foster, J. W., *J. Bact.*, 1944, **47**, 27.

of isolation it was possible to obtain purified crystals showing similar characteristics as those reported by Foster. It appears therefore that during the growth of tubercle bacillus riboflavin may be converted into lumichrome. This would furnish an explanation, at least partly, for the high incidence of riboflavin deficiency among tuberculous patients.

The finding that addition or increased concentration of riboflavin in the medium did not influence the growth of tubercle bacillus would lead us to believe that there is no danger of promoting the growth of the organism by therapeutic administration of riboflavin.

Summary. A slightly virulent strain of human tubercle bacillus was cultured in a vitamin free medium or in media to which riboflavin with or without other vitamins and biotin were added.

It was found that the presence or absence of riboflavin made no difference in the growth of the tubercle bacillus. On the other hand during the growth of the organism there was found a slow but steady disappearance of riboflavin from the medium. The presence of lumichrome crystals in the media containing riboflavin after growth of the tubercle bacillus indicated that the loss of the vitamin was due to conversion to lumichrome.

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17002

Recovery of Psittacosis Virus from Chicks Hatched from Inoculated Eggs.

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Investigations[1] of psittacosis infections in psittacine and other birds have shown that active infection with the virus (*Miyagawanella psittaci*) is encountered in young birds, even nestlings. This has suggested that infection is transmitted from latently infected adults either directly to the nestling or congenitally through the egg.[1] Furthermore the virus has been reported to have been recovered from ovaries and egg yolk in the oviduct of parakeets.[1] The possibility exists therefore, that congenital transmission may be important in dissemination of the virus among psittacine birds, pigeons, and other susceptible species.

In this laboratory an effort was made to secure experimental evidence concerning transmission of *M. psittaci* by the egg in avian species. Isolations of virus were attempted from chicks hatched from eggs which had been inoculated during various stages of embryonic development.

Although psittacosis infection in chickens is not known to be widespread this species has been found naturally infected[2] and the ready accessibility of chicken eggs and familiarity with technics for handling the infection in them made this species a logical choice for preliminary work.

The strain of virus used, No. 4, had been isolated in this laboratory from a psittacine bird (*Aratinga* sp.) and maintained by serial egg passage for at least 14 transfers. It was approximately 10^4 times more lethal for white mice by intracranial inoculation than by the intraperitoneal route and was well adapted to growth in allantoic cavity and yolk sac of the developing chick embryo.

[1] Meyer, K. F., *Medicine*, 1942, **21**, 175.

[2] Meyer, K. F., and Eddie, A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 522.