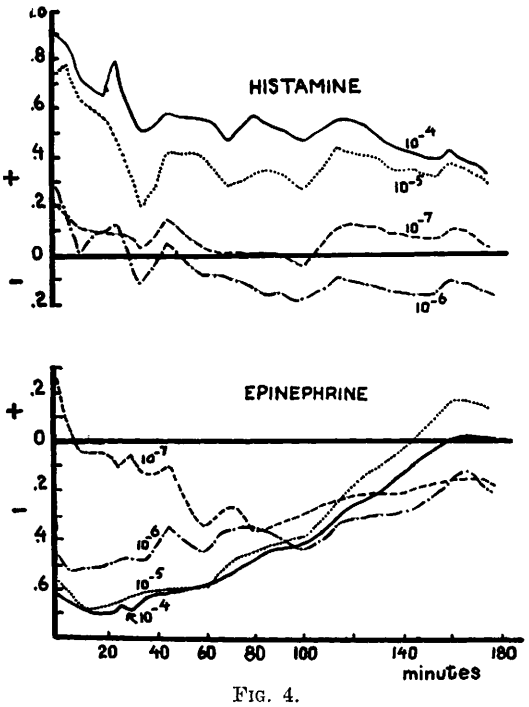


FIG. 4.

Fluorescein fluorescence time-intensity curves plotted as differences from the control saline wheal. Ordinate $= \frac{\text{fluorescence of drug wheal}}{\text{fluorescence of saline wheal}}$. + = relative hyperfluorescence; — = relative hypofluorescence.

ent form. The rate of disappearance of fluorescein from the blood and the intact normal skin of the rabbit are closely parallel,(4,6) indicating that the altered time-intensity dye curves produced by the administration of vasoactive drugs must be a property of altered capillary hemodynamics and/or a change in the permeability of the capillary wall. The exact factors responsible for the rate of dye exchange through the capillary wall can only be determined by the simultaneous measurement of these independent variables. The estimation of cutaneous fluorescein fluorescence as described above quantitatively indicates the gross rate of dye exchange across the capillary wall (neglecting lymphatic participation), but does not offer insight regarding the responsible specific factor(s).

*Summary.* 1. A quantitative method for measuring cutaneous fluorescein fluorescence is described.

2. The intensity of cutaneous fluorescein fluorescence is shown to parallel the expected capillary responses to various concentrations of vasoactive drugs.

3. The procedure described can be used to obtain in physiologically intact, unanesthetized experimental animals and probably human beings, a quantitative, sensitive and objective estimate of the cutaneous capillary circulation under a variety of experimental conditions.

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## Role of Vitamin $B_{12}$ in Reproduction of Poultry.* (17511)

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Research of the past few years showed that dried cow manure and certain animal protein supplements contained an unknown factor (or factors) required by chickens for growth and reproduction.(1,2) When crystalline $B_{12}$ became available, it was found to possess activity for chick growth equivalent to that of the unknown growth factor occurring in cow manure and liver extract.(3) The results

* Presented as part of symposium on vitamin $B_{12}$ and related factors, 116th meeting of American Chemical Society, September 22, 1949.

1. Rubin, M., and Bird, H. R., *J. Biol. Chem.*, 1946, v163, 387.

2. Whitson, D., Titus, H. W., and Bird, H. R., *Poultry Sci.*, 1946, v25, 143.

TABLE I.
Effect, on Hatchability, of Vitamin $B_{12}$ Injections in Hatching Eggs.

| Exp. | Class of embryo | Period of incub. at time of inj. | Level of $B_{12}$ inj. per egg,* μg | Type of injections: Hatchability of fertile eggs, None % | $H_2O$ % | $B_{12}$ % | Increase or decrease over $H_2O$ inj. eggs, % |
|---|---|---|---|---|---|---|---|
| 215 | — | 10 days | 1.25 | 70.0 | 47.8 | 71.4 | +23.6 |
| 220 | — | 10 " | 1.25 | 75.0 | 82.8 | 80.0 | — 2.8 |
| 228 | — | Prior to incub. | 0.50 | — | 74.4 | 83.3 | + 8.9 |
| 235 | 1 | 18 hr | — | 83.5 | — | — | |
| | 2 | " | 0.50 | — | 70.0 | 88.9 | +18.9 |
| | 3 | " | 0.50 | — | 0.0 | 80.0 | +80.0 |
| 240 | 1 | " | — | 86.6 | — | — | |
| | 2 | " | 0.50 | — | 64.1 | 77.2 | +13.1 |
| | 3 | " | 0.50 | — | 52.9 | 47.4 | — 5.5 |
| 247 | 1 | " | — | 90.7 | — | — | |
| | 2 | " | 0.50 | 83.3 | 68.9 | 76.9 | + 8.0 |
| | 3 | " | — | 45.9 | — | — | |
| 252 | 1 | " | — | 85.5 | — | — | |
| | 2 | " | 1.0 | 71.4 | 40.5 | 43.5 | + 3.0 |
| | 3 | 11 days | 1.0 | — | 43.8 | 71.4 | +27.6 |

* Injected solution contained 25 μg of vitamin $B_{12}$ per ml. An equivalent quantity of sterile distilled $H_2O$ was injected per egg in the $H_2O$-treated groups.

of investigation of the role of vitamin $B_{12}$ in reproduction are presented herewith.

*Materials and Methods.* Because of the limited quantities of crystalline vitamin $B_{12}$ available, the effect of the vitamin was tested by injection into eggs rather than by administration to hens. All the hatching eggs used were laid by Rhode Island Red hens fed a diet deficient in vitamin $B_{12}$.(4) The treated eggs were wiped clean at the large end (position of air cell) with cotton saturated with alcohol. A hole was drilled through the cleaned shell. Injections were made by means of a hypodermic needle into the albumen. Then the treated eggs were sealed with scotch tape and set in incubation trays. In all experiments there were control eggs injected with distilled water and in some experiments there were additional untreated controls. The number of eggs was limited by the supply of crystalline vitamin, and although the hens were selected on the basis of a previous record of low hatchability, the sample of eggs used in the second experiment hatched too well to be suitable material.

Thereafter, eggs were selected for injection according to a method developed at this laboratory(5) for identifying eggs of low potential hatchability after 18 hours of incubation. By this method eggs are divided into 3 classes. Hatchability of fertile eggs in classes 2 and 3 is about 20% and 40% less, respectively, than in class 1. In most cases, eggs segregated in this manner were injected immediately after segregation, that is, after 18 hours incubation; but in one case the class 3 eggs were injected after 11 days incubation to permit removal of the considerable number of infertile eggs included in this class.

At hatching time all the chicks were wing-pedigreed and fed a 70% soybean meal basal diet(6) with and without a vitamin $B_{12}$ concentrate. The chicks were reared in

3. Lillie, R. J., Denton, C. A., and Bird, H. R., *J. Biol. Chem.*, 1948, v176, 1477.

4. Rubin, Max, and Bird, H. R., *Poultry Sci.*, 1947, v26, 309.

5. Olsen, M. W., *Poultry Sci.*, 1949, v28, 731.

6. Bird, H. R., Rubin, Max, and Groschke, A. C., *J. Biol. Chem.*, 1948, v174, 611.

electrically heated metal batteries with raised screen floors. Feed and water were supplied *ad libitum*. Body weights were recorded weekly, and feather observations at the termination of the experiments. All growth tests were of 6 to 12 weeks duration.

At the time of feathering observations, a feather scoring system was employed whereby those chicks with normal feathers were given the score "O." Those whose feathers were slightly frayed, thus having a rough appearance, were given a score of 1. Those whose feather tips were normal, but with an intermediate portion of the rachis free of barbs, were given a score of 2. Those with brittle, broken feathers were given a score of 3.

*Results. Hatchability:* The data on the effect of vitamin $B_{12}$ injections on hatchability are summarized in Table I. Since hatching eggs of class 1, representing embryos of most advanced development, were characterized by high hatchability, none of these eggs was used for injection work. Due to the fact that a very small number of eggs of class 3 was available, none was used as a non-injected control. As indicated in Table I, in the last column, 7 out of a total of 10 lots of injected eggs responded favorably to $B_{12}$. Hence it is concluded that vitamin $B_{12}$ is required for hatchability of eggs laid by hens fed a diet deficient in the vitamin. Of the 3 lots that did not respond, one, composed of unsegregated eggs (Expt. 220), showed too high a hatchability without vitamin $B_{12}$ to permit any response, and another, the class 3 eggs in Experiment 240, constituted a small group in which the influence of unknown variables could easily have obscured the effect of the treatment.

*Growth.* The effects of $B_{12}$ injections in hatching eggs upon growth of chickens are summarized in Fig. 1. When the chickens were fed a basal diet deficient in the vitamin, all those that hatched from $B_{12}$-injected eggs grew at a rate superior to that of the $H_2O$-injected and non-injected groups for a period of 12 weeks. Since no differences were found between the $H_2O$-injected and non-injected groups, the results from these two groups were combined. Fig. 2 shows the effect upon chick growth of different methods of administration of vitamin $B_{12}$. All the experiments in this case were of 6 weeks' duration. The first two curves are comparable to those shown in Fig. 1. The second two curves show that the growth response was greater for those chicks that received the supplemented diet than for those fed the basal diet alone when all chicks were hatched from eggs not injected with $B_{12}$. As is shown in the remaining curves, the combination of dietary supplement with egg injection of the vitamin produced the greatest response.

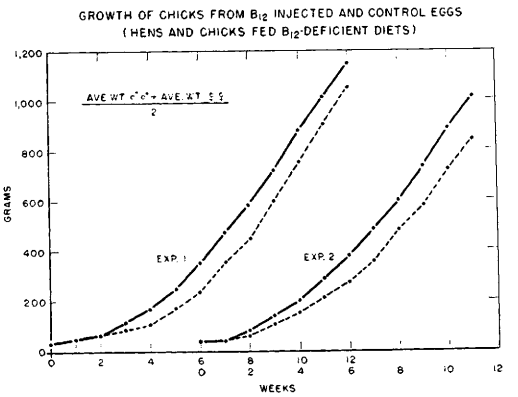


FIG. 1.

Growth of chicks from $B_{12}$-injected and control eggs. Hens and chicks were fed $B_{12}$ deficient diets. Solid line, chicks from $B_{12}$ injected eggs. Broken line, chicks from eggs not injected with $B_{12}$.

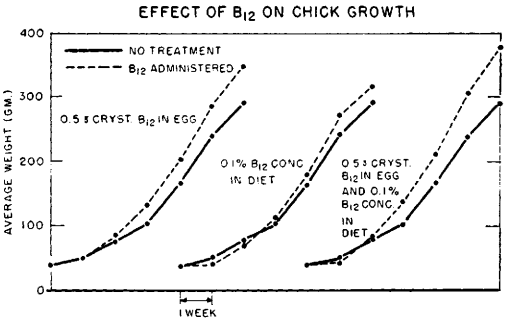


FIG. 2.

Effect on chick growth of vitamin $B_{12}$ injected into egg, added to diet, or administered by both routes.

*Viability and Feathering.* It was reported previously(7) that chicks hatched from the eggs of hens fed a diet deficient in the then un-

7. Bird, H. R., Rubin, Max, Whitson, Donald, and Haynes, S. K., *Poultry Sci.*, 1946, v25, 285.

TABLE II.
Effect of Vitamin $B_{12}$ Injections in Hatching Eggs Upon Viability and Feathering of Chickens.

| Exp. | Chick diet | Embryonic treatment | No. chicks | | Mortality at 6 wk, % | Chicks with abnormal feathering, % | Feather score* |
|---|---|---|---|---|---|---|---|
| | | | At start | At end | | | |
| 215 | Basal | $B_{12}$ | 30 | 22 | 26.6 | 36.3 | — |
| | | No $B_{12}$† | 34 | 13 | 61.7 | 84.6 | — |
| 220 | Basal | $B_{12}$ | 20 | 17 | 15.0 | 23.5 | 0.35 |
| | | No $B_{12}$† | 31 | 15 | 51.6 | 46.6 | 0.67 |
| 228 | Basal | $B_{12}$ | 18 | 11 | 39.9 | 27.3 | 0.55 |
| | | $H_2O$ | 16 | 14 | 12.5 | 38.5 | 0.77 |
| | + $B_{12}$ | $B_{12}$ | 17 | 11 | 35.3 | 9.1 | 0.09 |
| | | $H_2O$ | 16 | 9 | 43.8 | 44.4 | 0.55 |
| 247 | Basal | $B_{12}$ | 10 | 7 | 30.0 | 0.0 | 0.00 |
| | | $H_2O$ | 24 | 15 | 37.5 | 53.3 | 0.60 |
| | + $B_{12}$ | $B_{12}$ | 10 | 7 | 30.0 | 0.0 | 0.00 |
| | | $H_2O$ | 24 | 16 | 33.3 | 62.5 | 1.38 |
| 252 | Basal | $B_{12}$ | 17 | 14 | 17.6 | 21.4 | 0.21 |
| | | $H_2O$ | 12 | 9 | 25.0 | 66.6 | 1.00 |
| | + $B_{12}$ | $B_{12}$ | 15 | 11 | 26.6 | 27.2 | 0.45 |
| | | $H_2O$ | 12 | 10 | 16.6 | 30.0 | 0.60 |

* 0 = normal; 1 = slight abnormality; 2 = intermediate abnormality; 3 = extreme abnormality.

† Combined data from group receiving $H_2O$ injection and group receiving no injection.

known factor suffered a high mortality during the first week of life. This mortality occurred even when the chicks were fed a supposedly complete diet but was corrected by correcting the deficiency in the hens' diet. It has also been observed that the progeny of hens fed such a deficient diet show abnormal feathering which is not corrected by adding supplements to the chicks' diet.

The effects of $B_{12}$ injections in hatching eggs upon the viability and feathering of such chickens are summarized in Table II. With two exceptions, the mortality was greater for chicks hatched from $H_2O$-injected and non-injected eggs than for those hatched from $B_{12}$ injected eggs, irrespective of chick diet. In experiments 215 and 220, in which 1.25 micrograms were injected on the tenth day of incubation, the $B_{12}$ injection reduced mortality very materially. Feathering of chicks hatched from $B_{12}$-injected eggs was superior to feathering of those from the $H_2O$-injected and non-injected eggs. As in earlier experiments addition of the vitamin to the diet of chicks hatched from deficient eggs was not effective in improving feathering.

*Discussion.* The data summarized here indicate the importance of vitamin $B_{12}$ in reproduction of poultry, not only from the standpoint of hatchability but also from the standpoint of growth, viability and feathering of progeny. The effectiveness of a single dose of 0.5 to 1.25 $\mu$g of $B_{12}$ injected into hatching eggs in enabling the progeny to maintain an advantage in growth over control chicks for a period of 12 weeks is remarkable and indicates the high potency of the vitamin per unit of weight. The fact that injection of vitamin $B_{12}$ into deficient eggs reduced the mortality and improved the feathering of chicks and that these results were not achieved by supplementing the chicks' diet reemphasizes the importance of maternal diet and shows that deficient maternal diet may lead to irretrievable post-natal damage as well as pre-natal effects. Although vitamin $B_{12}$ reduced mortality and improved feathering, it was not completely effective with respect to either. This might well have been due to the very limited dosage, but of course there exists

the possibility that other dietary factors are involved.

*Summary.* When hatching eggs laid by Rhode Island Red hens fed a diet deficient in vitamin $B_{12}$ were injected with crystalline vitamin $B_{12}$, the hatchability was improved. Among the chicks hatched from vitamin $B_{12}$-injected eggs, the growth rate was greater, the mortality lower and the feathering better than among chicks hatched from $H_2O$-injected and non-injected eggs.

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## Distribution of Chloramphenicol (Chloromycetin*) and its Metabolic Products Between Human Red Cells and Plasma. (17512)

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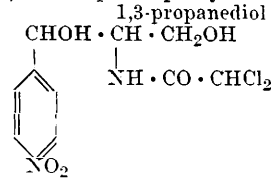
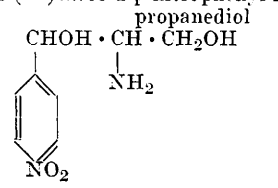
Following the administration of chloramphenicol to human subjects, 3 aromatic nitro compounds were identified as excretory products in the urine.(1) These were unchanged chloramphenicol (I), an amino diol produced from chloramphenicol by hydrolysis (II), and a conjugate of chloramphenicol with glucuronic acid (III):

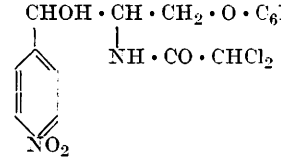
I. Chloramphenicol
D(—)*threo*-1-p-nitrophenyl-2-dichloracetamido-1,3-propanediol



II. Hydrolysis product
D(—)*threo*-1-p-nitrophenyl-2-amino-1,3-propanediol



III. Glucuronide derivative.



Of these compounds only I showed a significant degree of antibiotic activity. The glucuronide derivative III constitutes the major excretory product in human urine, although small amounts of I and II are also present.(1,2) The reduction of the nitro group is negligible in man, although considerable quantities of aryl amines may appear in the urine and feces of lower animals.(2) The data presented here summarize our observations on the distribution of I, II and III between the red cells and plasma of heparinized human blood.

*Procedure.* The chloramphenicol used in these experiments was a crystalline product isolated from fermentation sources.† The amino diol was prepared by hydrolysis of chloramphenicol,(3) and the glucuronide derivative was isolated from human urine in this laboratory.(1) Blood drawn from the antecubital veins of normal human subjects was treated with a few milligrams of powdered heparin (110 units per mg) to inhibit coagulation, and was used immediately. In a typi-

* Parke, Davis and Company trademark for chloramphenicol.

1. Glazko, A. J., Dill, W. D., and Rebstock, M. C., *J. Biol. Chem.*, 1950, in press.

2. Glazko, A. J., Wolf, L. M., Dill, W. D., and Bratton, A. C., *J. Pharm. Exp. Therap.*, 1949, v96, 445.

† The chloramphenicol used in these experiments was kindly supplied to us by Dr. John Ehrlich and Dr. Fred Stimpert. The amino diol (II) was prepared from chloramphenicol by Dr. Mildred Rebstock and Dr. Harry M. Crooks.

3. Rebstock, M. C., Crooks, H. M., Controulis, J., and Bartz, Q. R., *J. Am. Chem. Soc.*, 1949, v71, 2458.