of 5 patients, at the time when virus was obtained from the buboes. The virus of lymphogranuloma venereum was isolated from both blood and spinal fluid of one patient (No. 161), a 20-year-old woman, tested on the sixth day of the disease. The agents from her blood and spinal fluid produced signs of infection in chick embryos on the first passage, whereas material from her bubo required one blind passage before infection was evident. From 8 to 17 passages were made on all 3 agents. Elementary bodies were demonstrated in spreads of infected mouse brains and yolk sacs, and the tissue tropisms and susceptibility to sulfonamide therapy, characteristic of the virus of lymphogranuloma venereum, were found to be properties of these agents. In addition, serologic identification of the viruses was established by neutralization of their toxins by hyperimmune serum prepared in chickens against a known strain (J.H.) of the virus of lymphogranuloma.[4,6,7]

*Summary.* Eight patients with acute lymphogranuloma venereum, proved by isolation of the virus from buboes, were examined for the presence of virus in blood or spinal fluid, or both. The virus was found in the blood and also in the spinal fluid of one patient. The clinical features in this case were not significantly different from those of the other 7 cases. This is the first reported instance of isolation of the agent of lymphogranuloma venereum from the blood of a human being.

[6] Rake, G., and Jones, H. P., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 86; *J. Exp. Med.*, 1944, **79**, 463.

[7] Hilleman, M. R., *J. Infect. Dis.*, 1945, **76**, 96.

## 15461

## Effect of Diet on the Response of Chicks to Folic Acid.*

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Previous studies[1] in our laboratory indicated that chicks showed less growth, feather development and hemoglobin production when preparations of impure folic acid (obtained from Texas University) and the third *L. casei* factor (obtained from Lederle Laboratories, Inc.) were added to our basal ration containing sulfasuxidine than when added to the basal ration alone. Scott *et al.*[2] found the third *L. casei* factor to be somewhat active in the presence of sulfasuxidine, particularly in the presence of the pyridoxic acids. Subsequent work[3] showed that the activity of synthetic folic acid is apparently decreased when it is fed in diets containing sulfasuxidine. In an attempt to determine the function of folic acid by measuring its activity under different dietary conditions it was found that changes in diet may markedly alter the response observed when a given amount of folic acid is fed to chicks.

*Experimental.* Day-old White Leghorn chicks weighing between 40 and 50 g were divided uniformly into groups of 10, placed in electrically-heated cages with raised screen bottoms and fed the diets described in Table I. It is important to note that the level of vitamins was considerably higher than that normally used. The largest and smallest

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[1] Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1945, **158**, 303.

[2] Scott, M. L., Norris, L. C., Heuser, G. F., and Bruce, W. F., *J. Biol. Chem.*, 1945, **158**, 291.

[3] Luckey, T. D., Moore, P. R., Elvehjem, C. A., and Hart, E. B., *Science*, 1946, **103**, 682.

TABLE I.
Composition of Basal Diets.

| Constituent | 494 | 504 | 505* | 506 | 507 | 508 | 509 | 510 | 512 |
|---|---|---|---|---|---|---|---|---|---|
| Dextrin | 61 | 39 | 59 | | | | | 46 | |
| Sucrose | | | | 61 | | | | | 46 |
| Glucose | | | | | 61 | | | | |
| Starch | | | | | | 61 | | | |
| Corn meal | | | | | | | 67 | | |
| Casein† | 18 | 50 | | 18 | 18 | 18 | 12 | 18 | 18 |
| Egg albumin | | | 30 | | | | | | |
| Gelatin | 10 | | | 10 | 10 | 10 | 10 | 10 | 10 |
| Cystine | .3 | | | .3 | .3 | .3 | .3 | .3 | .3 |
| Salts | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Soybean oil | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 20 | 20 |
| Folic acid content/100 g‡ | 4 | 6 | 44 | 1 | 2 | 5 | 17 | 4 | — |

Vitamins per 100 g: A, 2400 I.U.; $D_3$, 240 I.U.; E, 0.6 mg; K, 0.1 mg; thiamine Cl, 0.6 mg; riboflavin, 1.2 mg; Ca pantothenate, 4 mg; choline Cl, 300 mg; nicotinic acid, 10 mg; pyridoxine Cl, 0.8 mg; biotin, 0.04 mg; and inositol, 200 mg.

* 200 μg of biotin per 100 g diet were added to counteract the avidin present in this diet.

† Crude casein was extracted by stirring 3 times with 1½ volumes of 95% alcohol for a total of 6 hours at 78°C.

‡ As measured with *S. faecalis*[5] after acid hydrolysis.[6]

chicks in each group were discarded at the end of the first and at the end of the second weeks (with the one exception noted) and the experiment was terminated when the remaining 6 chicks were 4 weeks old. The methods used for determining hemoglobin values and measuring changes in feather development were the same as those used previously.

*Results. Effect of Protein.* The data in Fig. 1A show that good hemoglobin and growth responses and normal feather development were obtained when 50 μg of folic acid per 100 g of ration was added to the regular dextrin diet. When the casein in the diet was increased to 50% (Fig. 1B), a low level of folic acid produced a striking response in growth and hematopoiesis, but the best growth obtained with this diet did not approach that obtained with the lower level of casein. With the high casein diet the feather development was not normal even when high levels of folic acid were fed. When the protein of the ration was replaced with 30% commercial egg albumin (Fig. 1C), growth was poor and the folic acid did not improve the rate of growth or hemoglobin production. This level of egg albumin supplied 40 μg of folic acid per 100 g of basal ration and this intake was undoubtedly sufficient to give the response observed without any added folic acid. The fact that growth with this diet was better than that obtained with the high level of casein and the fact that no dermatitis was observed indicate that the biotin added was adequate to counteract the avidin supplied by the egg albumin.

*Effect of Carbohydrate.* When sucrose was substituted for dextrin (Fig. 1D), the folic acid supplements produced little response until a level of 50 μg was used and maximum responses were not obtained until 100 μg were added. These results are in marked contrast to those obtained when dextrin was the carbohydrate. When glucose was substituted for dextrin (Fig. 1E), 20 μg of folic acid per 100 g of ration produced a growth response but allowed little feather development. Normal feathers were not obtained until 100 μg of folic acid was fed. The maximum growth attained with glucose as well as with sucrose was considerably less than that obtained with dextrin. Feather development was also irregular when low levels of folic acid were fed in the diet containing starch (Fig. 1F). The feathers of chicks receiving 10 μg of folic acid were less developed than those of chicks receiving no folic acid although the growth was 30 g better in the former chicks.

[5] Luckey, T. D., Briggs, G. M., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, 1944, **152**, 157.

[6] Luckey, T. D., Briggs, G. M., Jr., Moore, P. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1945, **161**, 395.

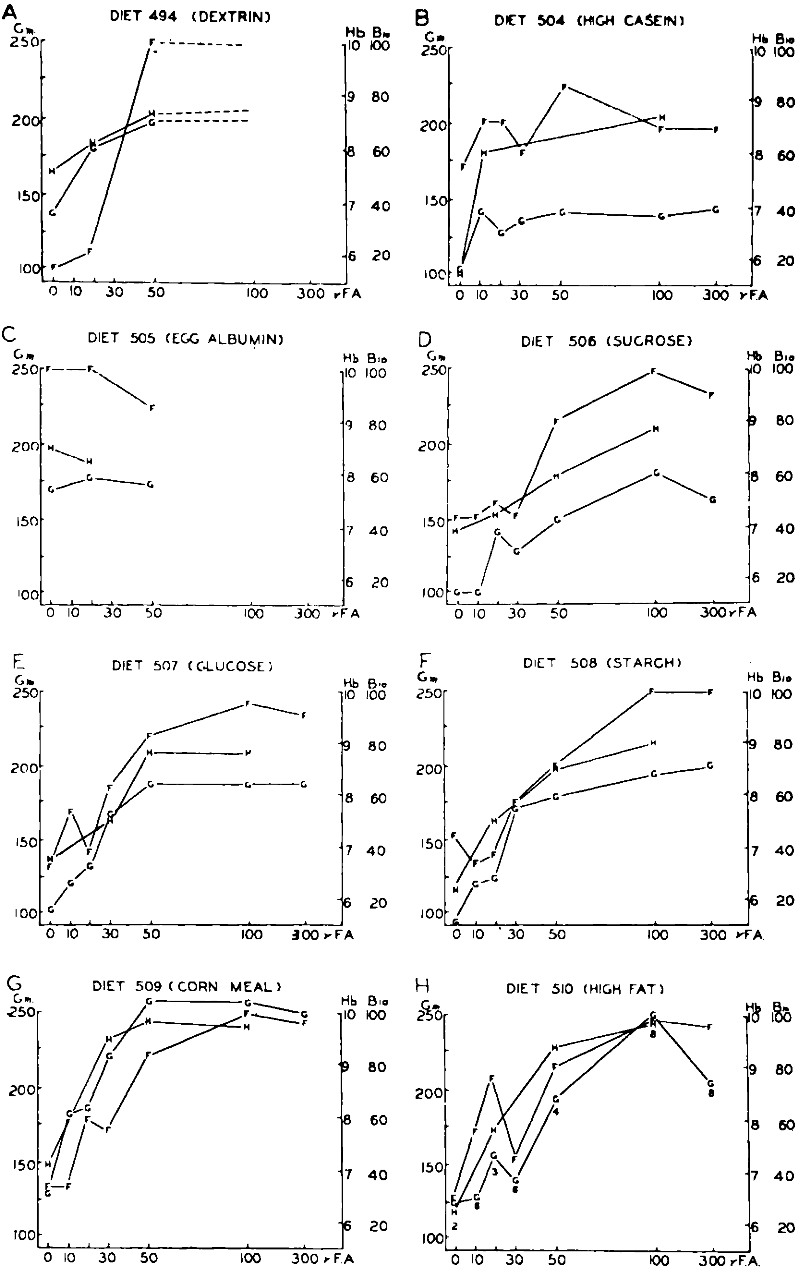


Fig. 1.

The responses in growth, feather development, and hematopoiesis observed in 4-week-old chicks fed different basal diets and 0-300 $\mu$g of folic acid per 100 g of diet.

Gm = Average weight of the 6 chicks in each group at 4 weeks and is indicated by the letter G.

Hb = Average grams of hemoglobin per 100 cc of blood for 4 chicks in each group and is indicated with the letter H.

$B_{10}$ = Average feather development and regression for each group of chicks and is indicated with the letter F. O = very poor feathers and 100 = very good.

The numbers in Fig. 1H indicate the number of chicks out of 8 that survived on that diet. Chicks on diets other than No. 510 died only in those groups receiving no folic acid. The number dead for each basal group is: No. 494—0, No. 504—2, No. 505—0, No. 506—3, No. 507—3, No. 508—2, No. 509—0.

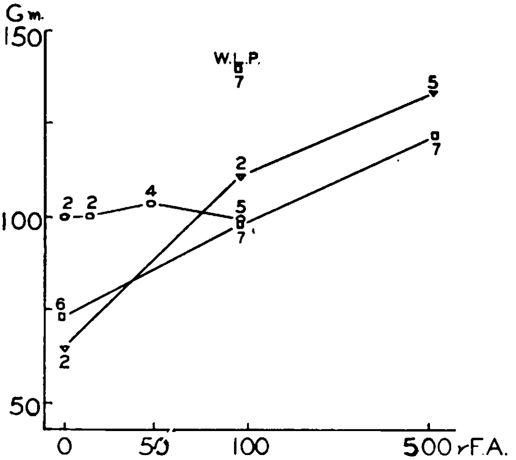


Fig. 2.

Comparison of the effect of the dextrin-high fat, sucrose-high fat, and sucrose-normal fat diets upon the response of chicks at 3 weeks to different amounts of folic acid and liver.

○ = Diet No. 510 (dextrin-high fat).
△ = Diet No. 512 (sucrose-high fat).
□ = Diet No. 506 (sucrose-normal fat).

The average weight of each group of 7 chicks is plotted against the γ of folic acid per 100 g of diet. The number of chicks surviving 3 weeks is given for each group. The group designated W.L.P. was fed Diet No. 506 + 5% whole liver powder (≐ 100 γ folic acid) + 100 γ folic acid.

When cornmeal was fed at 69% of the diet (Fig. 1G), 10 μg of folic acid per 100 g of diet produced a good growth response but had little effect on the feather development. When 50 μg of folic acid was added feather development still was not as complete as on the dextrin diet. The hemoglobin values obtained with this diet were higher than those obtained with the other diets.

*Effect of Fat.* When 20% soy bean oil was included in the basal diet containing dextrin as the carbohydrate (Fig. 1H), a marked feather response to low levels of folic acid was noted while only small growth responses were obtained with levels of folic acid which had previously been adequate. Thus a comparison of the basal group with the group receiving 10 μg of folic acid per 100 g of diet shows essentially no difference in weight but a definite difference in feather development. Evidently under this regimen folic acid fulfilled the requirement for vitamin $B_{10}$ but not for vitamin $B_{11}$. The growth and hemoglobin values obtained in this series were higher than those obtained when a soluble carbohydrate was fed. Since several chicks died during the second week no chicks were discarded at the end of that week, so the results for this series are based upon 8 chicks per group. From the mortality data indicated in the figure it is evident that high levels of folic acid were effective in counteracting this condition.

In a second experiment, in which 7 chicks weighing 38-42 g were started in each group, the results at the end of 3 weeks indicated again (Fig. 2) that folic acid reduced the high mortality observed on the dextrin, high-fat diet. A growth response with added folic acid was not observed. When sucrose was used as the carbohydrate (diet No. 506) the results indicated that extremely high levels of folic acid might be required to produce maximum growth while 5% of whole liver powder in the diet produced a growth response which could not be accounted for by its folic acid content. This effect has also been observed with a dextrin diet.[3] Whether it is due to a known vitamin, amino acid or a new factor has not been determined. When the sucrose high-fat diet (No. 512) was fed, 500 μg of folic acid per 100 g of diet did not completely counteract the mortality. All chicks receiving the high-fat diets had an extensive dermatitis similar to that reported by Ben-Ami Ben-Dor,[4] but this does not seem to be a lethal dermatitis under the conditions of our experiments.

*Discussion.* When low levels of folic acid were used in purified diets, there appeared to be no direct relationship between the growth response and the feather development. With high-casein diets no correlation was observed between feather development and the amount of folic acid in the diet. More than 30 μg of folic acid per 100 g of diet was needed to produce a feather response with the sucrose diet, while a marked feather response with low levels of folic acid may be seen when dextrin is fed with either 5 or 20% fat. Growth responses with low levels of folic acid were good on diets containing dextrin, starch, glucose, and corn meal (except the high fat-dextrin diet) and poor with the sucrose and

4 Ben-Dor, B., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 495.

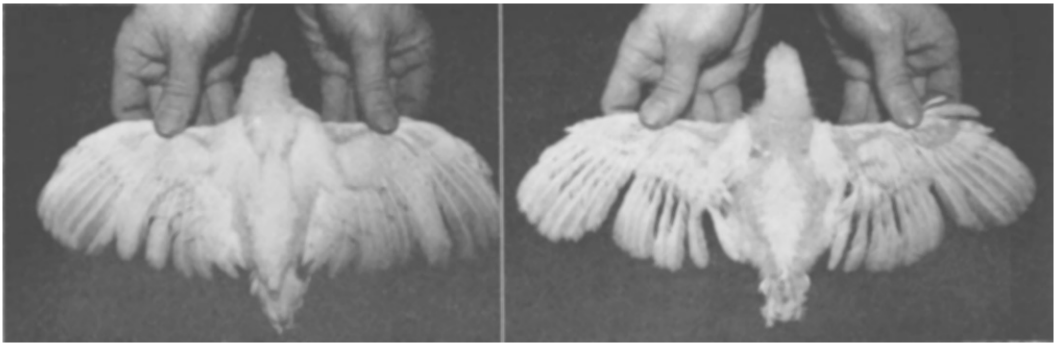

Fig. 3.

Typical results showing differences in the feather development in chicks growing at approximately the same rate. The data for the groups of chicks represented by 2 typical chicks above are:

| No. 494 (dextrin) | Diet | No. 509 (corn meal) |
|---|---|---|
| 50 | $\gamma$ folic acid per 100 g diet | 30 |
| 100 | feather development ($B_{10}$) | 55 |
| 200 g | weight | 220 g |

high-fat diets.

Lack of correlation between feather development and rate of growth was observed on the high-casein, glucose, starch and corn-meal diets when the folic acid content was low. Although the differences are not large, they are significant in view of the fact that variation was minimized by starting with chicks of uniform weight which were hatched together and raised at the same time in one set of batteries. The uniformity was improved by discarding 40% of the large and small chicks early in the experiment. A typical difference in feathering is given in Fig. 3. The chick on the left, which received the dextrin diet + 50 $\mu$g of folic acid per 100 g of diet, weighed somewhat less and feathered much better than the chick on the right which was raised for 4 weeks on the corn-meal diet + 30 $\mu$g of folic acid per 100 g of diet. This lack of correlation indicates the existence of 2 compounds fulfilling these functions. It is interesting to note that most of the work with vitamins $B_{10}$ and $B_{11}$ was carried out with low levels of folic acid (3-25 $\mu$g per 100 g of diet).

These differences may be due: to variations in the utilization of the added folic acid by chicks in the different groups; to variations in possible reactions of the folic acid with the other nutrients or metabolites; or to an indirect action of folic acid on the intestinal flora whereby the production of vitamin $B_{10}$ (the feather factor) or vitamin $B_{11}$ (the growth factor) would be increased. It seems very unlikely that one compound could give these different responses unless it caused the production or affected the utilization of more than one nutrient.

The great variation in response obtained from a given level of folic acid under different conditions indicates that no definite requirement for this compound can be made without specifying the type of diet. Thus although 10 $\mu$g of folic acid per 100 g of diet gave maximum growth when fed with a high-protein diet, maximum growth was not attained with a diet containing sucrose as the carbohydrate or 20% fat until the level of folic acid was 100 $\mu$g per 100 g of diet. Whether this is evidence for the indirect action of folic acid or an indication of the biological function of this compound needs further study.

In general 3 types of responses seem to be obtained with these different diets (Fig. 4). The anemia is the most severe with the starch or high-casein diets and is the most readily counteracted with small amounts of folic acid. The other extreme is seen with the diets containing sucrose or glucose as the carbohydrate. In these cases the anemia is not severe and the response to folic acid is slow. An intermediate response to low levels of folic acid

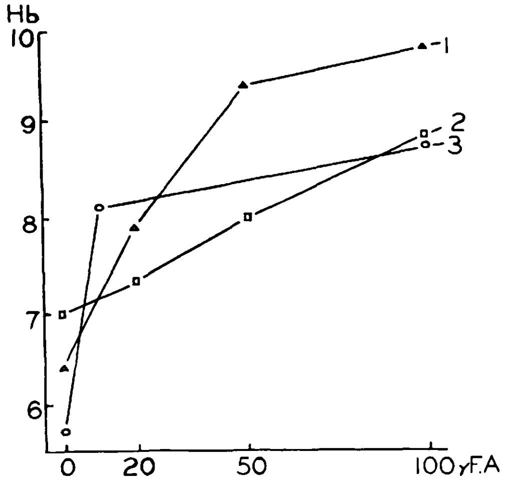


Fig. 4.

Summary of Hemoglobin Production.

The hemoglobin values (in grams per 100 cc of blood) are plotted against the μg of folic acid per 100 g of diet.

1 = High-fat diet (the curves obtained with the corn meal diet or the regular dextrin diet are similar).

2 = Sucrose diet (this is similar to the curve obtained with glucose as the carbohydrate).

3 = High-casein diet (the starch diet produced a similar curve).

is obtained with the corn-meal or dextrin (high or low fat) diets but higher levels of folic acid produce more hemoglobin than is obtained with the other diets. Since low levels of folic acid are effective when fed with high-protein diets and ineffective in diets containing high fat, glucose or sucrose, it seems evident that treatment of this type of anemia should be facilitated by high-protein, low-fat, and low-soluble carbohydrate diets.

The reason for the superior growth and hemoglobin production with a corn-meal diet is not known, but it is evident that corn is, when properly supplemented, a good dietary constituent.

*Summary.* A study of the effects of dietary constituents upon the activity of synthetic folic acid in chick nutrition shows that no definite requirement for this compound can be established since the response to a given amount of folic acid depends upon the type of ration used. Folic acid produced the least response with high-fat diets or diets containing glucose, sucrose, or starch as the sole carbohydrate, and the best response with diets containing high protein, low fat, or corn-meal and dextrin as the carbohydrates.

Hemoglobin responses were better on diets containing dextrin or corn-meal than diets containing other carbohydrates.

Feather development cannot be correlated with either the amount of folic acid in the diet or the rate of growth of the chicks when low levels of folic acid are included in the diet.

Whole liver substance when added to a diet containing sucrose as the carbohydrate gave an increased growth response that could not be attributed to its folic acid content.

We thank Merck and Company, Inc., Rahway, N.J., for the crystalline vitamins, Wilson Laboratories, Chicago, Ill., for gelatin and whole liver powder, Allied Mills, Peoria, Ill., for soy bean oil, and Lederle Laboratories, Inc., Pearl River, N.Y., for the folic acid.

## 15462

# Effect of Some of the B Complex Vitamins upon Chick Tissue Cultures.

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In view of the prominence which some of the vitamins of the B Complex are assuming in the role of therapy, especially in some disorders resulting in alterations of the skin and of the nervous system, it was thought of interest to discover whether or not some