increased logarithmically with increasing levels of PGA to as high as 200 μg per 100 g of diet.

The concentrations of PGA in the livers of the chicks also provide some evidence concerning the mode of action of the vitamin in reducing the apparent liver xanthine oxidase activity. *In vitro* tests of the sample of PGA used in these experiments indicated that a concentration of approximately 40 μg per ml would be needed to reduce the xanthine oxidase activity to one-half its initial value.† The relatively low levels of PGA encountered in all of the liver samples indicate that either the impurity probably responsible for the *in vitro* anti-xanthine oxidase activity[16] is better retained than is PGA, or that some other mechanism than the accumulation of this substance is responsible for the reduced activity found in our previously published experiments.[1] It is conceivable that the xanthine oxidase is inhibited or reduced by a metabolic product of PGA normally produced in relatively large quantity.

Summary. Determinations of desoxypentose nucleic acid and pentose nucleic acid, pteroylglutamic acid (PGA), and conjugase have been made on the livers of chicks receiving 0, 5, 10, 20, 40, 80, 200, and 1000 μg of PGA per 100 g of diet and on controls receiving a commercial diet. Desoxypentose nucleic acid was found to be somewhat low in the livers of the negative control group of chicks but differences between all other groups were of doubtful significance.

The liver conjugase levels of the various groups, whether determined at pH 4.5 or at pH 7.0, were found to be unrelated to the dietary intake of PGA and to the nucleic acid content of the livers.

The liver PGA freed by autolysis was approximately the same as that determinable after treatment with either chick pancreas conjugase or hog kidney conjugase. The levels of PGA found in the chick livers indicate that storage of excess vitamin did not take place until the dietary level exceeded 40 μg per 100 g.

The bearing that the findings have on the mode of action of dietary PGA in reducing liver xanthine oxidase is briefly discussed.

[14] Campbell, C. J., McCabe, M. M., Brown, R. A., and Emmett, A. D., *Am. J. Physiol.*, 1945, **144**, 348.

[15] Unpublished data from this laboratory.

† Experiments conducted *in vitro* indicate that 2 mols of the xanthine oxidase inhibitor react with one mol of chick liver xanthine oxidase while only one mol reacts per mol of rat liver xanthine oxidase. That is, the degree of inhibition using chick liver is proportional to the square of the concentration of added PGA while it is proportional to the logarithm of the PGA concentration when using rat liver as the source of xanthine oxidase.

[16] Kalekar, H. M., Kjelgaard, N. O., Klenow, H., *J. Biol. Chem.*, 1948, **174**, 771.

16953 P

Influence of Pteroylglutamic Acid Administration Upon Fecal Riboflavin Values in Human Subjects.*

LURA MAE ODLAND, DOLORES M. OTTO,† AND HELEN T. PARSONS.

From the Department of Home Economics, College of Agriculture, University of Wisconsin, Madison.

In the course of investigations in this laboratory on the metabolism of riboflavin in human subjects on weighed, uniform diets of

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation; and by a commercial grant from the Jos. Schlitz Brewing Company of Milwaukee.

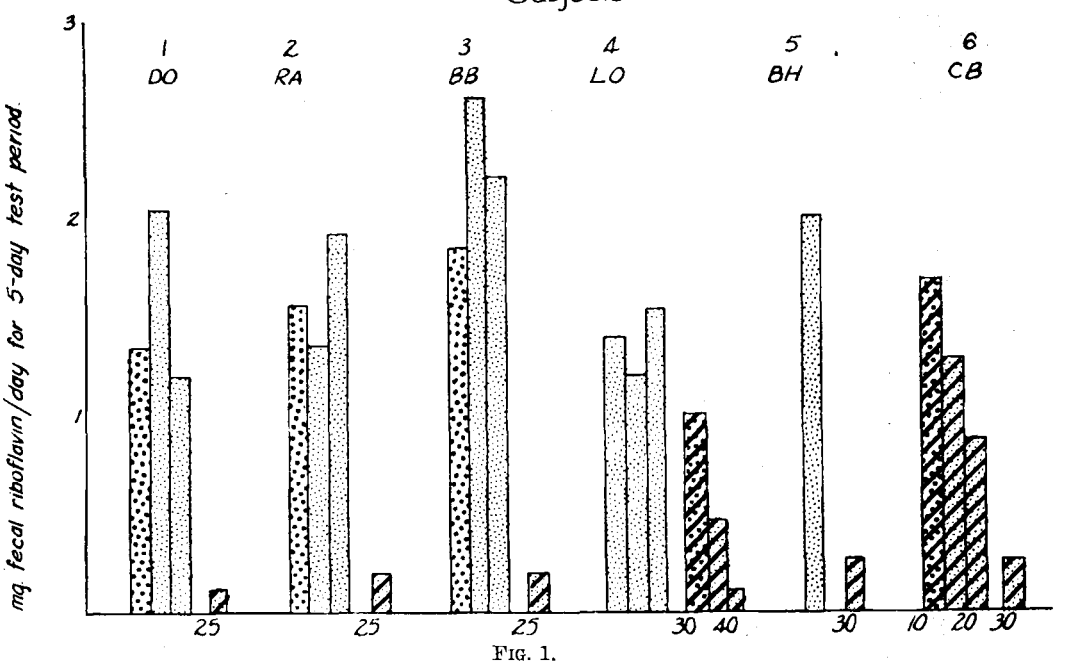

FIG. 1.

The progressively depressing effect of the daily administration to human subjects of 10 mg doses of pteroylglutamic acid on fecal riboflavin values.

Small dots: basal diet; larger dots: self-chosen diet; ////: 10 mg dose pteroylglutamic acid; numerals on the abscissa indicate number of days dose prior to fecal collection.

known riboflavin content[1-3] doses of pteroylglutamic acid were incorporated in certain of the experimental diets.

Experimental. Six university women in a satisfactory nutritional state were maintained on an adequate uniform weighed basal diet providing a daily intake of 3.8 mg of riboflavin with and without the addition of 10 mg of synthetic pteroylglutamic acid. This dose is considered to be within the lower part of the therapeutic range.[4] For the first two basal periods of Subject 4, the diet was identical with one reported earlier[1] and for all other subject-periods this diet was modified only by the addition of 50 g of meat loaf, thus increasing the protein content of the diet to 84 g. For securing additional data, fecal riboflavin values for 5-day periods were also determined on self-chosen diets.

For conformity with other studies, certain supplements were added to the basal diet‡ as follows: Subjects 1, 2 and 4 received 14 g dried brewers' yeast daily, and Subjects 3, 4 and 6 received supplements‡ of 2.0 mg thiamine, 0.45 mg riboflavin, 5.24 mg niacin, 50.4 mg choline, 0.14 mg pyridoxine, 1.0 mg calcium pantothenate, and 10 μg pteroylglutamic acid with the exception of the first 2 periods of Subject 4.

Five-day fecal collections were homogenized in a Waring Blendor and preserved in acid-alcohol. Riboflavin was determined by the fluorometric method of Conner and Straub.[5] Duplicate determinations were made

† Present address: Department of Home Economics, University of California, Berkeley, Calif.

1 Price, E. L., Marquette, M. M., and Parsons, H. T., *J. Nutrition*, 1947, **34**, 311.

2 Price, E. L., M. S. Thesis, 1946, Univ. Wis.

3 Otto, D. M., M. S. Thesis, 1948, Univ. Wis.

4 Spies, T. D., *Annual Rev. Biochem.*, 1948, **17**, 449.

‡ Thanks are due to the Lederle Laboratories, Inc., Pearl River, N. Y., for the synthetic pteroylglutamic acid (Folvite); to Hoffman La Roche, Nutley, N. J., for other vitamin supplies; and to the Pineapple Research Institute of Hawaii for the gift of specially packed crushed pineapple.

5 Conner, R. T., and Straub, G. J., *Ind. Eng. Chem. Anal. Ed.*, 1941, **13**, 385.

on separate days for all samples. Food aliquots representing 25% or 50% of the weighed daily food intake, except the self-selected diets, were homogenized and assayed for riboflavin.

Results and discussion. The fecal riboflavin values observed during the control periods are consistent with other data from this laboratory for subjects ingesting essentially the same basal diet. Similar fecal riboflavin concentrations are also reported by Hathaway and Lobb[6] for subjects ingesting a natural diet containing 1.33 mg of riboflavin.

Upon supplementation of the diets of the subjects with 10 mg of pteroylglutamic acid daily for periods of 25 to 40 days, regular and consistent decreases were observed in fecal riboflavin. Values comparable to the lower range in this series have been noted in this laboratory only in one other instance, *i.e.*, when subjects were consuming a diet composed of milk with ascorbic acid and mineral supplements.[7]

Darby *et al.*[8] have noted that certain deficiency states due to decreases in gastrointestinal absorption may be relieved by the administration of pteroylglutamic acid. These observations suggest that in the present experiment, certain vitamin interrelationships involving the absorption of riboflavin might be concerned in the decrease in fecal riboflavin in the presence of long-continued daily dosage of 10 mg of pteroylglutamic acid. Another obvious possibility is that administration of pteroylglutamic acid measurably influences the character of the intestinal flora, resulting in a decrease in riboflavin synthesis, an increase in riboflavin destruction or both. The nature of this pronounced change in fecal riboflavin is being investigated; these results are being reported at this time because of their possible clinical implications.

Summary. As a part of long-time experiments in this laboratory on riboflavin metabolism in human subjects on weighed diets of known riboflavin content, it was noted that with the daily administration of 10 mg of synthetic pteroylglutamic acid for 25 to 40 days there was a striking decrease in fecal riboflavin values as compared to basal periods.

[6] Hathaway, M. L., and Lobb, D. E., *J. Nutrition*, 1946, **32**, 9.

[7] Gardner, J., Neal, A. L., Peterson, W. H., and Parsons, H. T., *J. Am. Dietet. Assn.*, 1943, **19**, 683.

[8] Darby, W. J., Jones, E., Warden, H. F., and Kaser, M. M., *J. Nutrition*, 1947, **34**, 645.

16954

Effect of Adrenal Cortical Extract on the Blood Picture and Serum Proteins of Fowl.

ANSELL B. SHAPIRO AND A. M. SCHECHTMAN.
From the Department of Zoology, University of California, Los Angeles, Calif.

Preliminary to studies on the effects of adrenal cortical extract (ACE) on embryonic development of the chick's blood, it became desirable to know how the blood elements of the adult fowl respond to cortical substances. We have been unable to find pertinent data for the fowl; furthermore the results obtained with various mammals are not consistent. Absolute peripheral lymphopenia has been observed in the normal mouse,[1] rat,[1,2,3] rabbit,[1] dog,[2] and in man[4,5] after injections of pituitary adrenotrophic hormone, and in the mouse,[1] rat,[1,6] rabbit,[1] and man[1,4,5] after

[1] Dougherty, T. F., and White, A., *Endocrinology*, 1944, **35**, 1.

[2] Reinhardt, W. O., Aron, H., and Li, C. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **57**, 19.

[3] Yoffey, J. M., and Baxter, J. S., *J. Anat.*, 1946, **80**, 132.

[4] Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G., *J. Clin. Endocrinology*, 1948, **8**, 15.