the severity of perosis many of the chicks were unable to secure food and water and died before the end of the 6-week experimental period. As a result the 6-week weights and hemoglobin values of the first 2 lots on the sulfonamide diet are somewhat lower than the corresponding lots on the non-sulfonamide diet.

The feather scores indicated that succinylsulfathiazole included at 2% of the basal diet had no detrimental effect on feathering. The feather score in the basal lot of Experiment 2(b) was not considered typical since it represented the scores of only 2 chicks. There was no difference in the feather scores obtained on the 2 diets indicating that folic acid *per se* is concerned with feathering and that intestinal synthesis of a feather factor is not probable. The requirement of folic acid for good feather development appears to be not less than 55 $\mu$g per 100 g of diet.

Survival of chicks to 6 weeks of age in Experiment 2 required approximately 25 $\mu$g of folic acid per 100 g of diet. Data from Experiments 1 and 3 confirm this estimate.

*Summary.* The folic acid requirement of chicks for growth, feathering, and hemoglobin formation has been determined using a purified diet containing 2.0 $\mu$g of preformed folic acid per 100 g of diet. The total folic acid content of the basal diets as determined microbiologically following incubation with chick liver was 5 $\mu$g per 100 g of diet for Experiment 1 and 15 $\mu$g for Experiments 2 and 3.

The following amounts of folic acid, which include the amount in the basal diet, were required for its several functions: for survival to 6 weeks of age, approximately 25 $\mu$g per 100 g; for growth and hemoglobin formation at 4 weeks of age, 45 $\mu$g; for growth at 6 weeks, 45 $\mu$g, but for hemoglobin formation at 6 weeks, 35 $\mu$g; and for feathering at 6 weeks of age, not less than 55 $\mu$g per 100 g of diet.

In these experiments the addition of succinylsulfathiazole at 1 or 2% of the diet did not have any effect on growth, feathering, or hemoglobin formation in chicks to 6 weeks of age.

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## Milk as a Source of the Monkey Anti-Anemia Factor.*

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Previous studies[1,2] have shown that whole liver powder, raw and lyophilized liver are good sources of the monkey anti-anemia factor. In this paper we wish to report experiments carried out to determine if milk contains this factor.

*Experimental.* The method of handling the monkeys has been described previously.[3] The assay monkeys consisted of animals which failed to show complete recovery from a riboflavin deficiency after riboflavin therapy,[2] and animals made deficient in the monkey anti-anemia factor by the prolonged feed-

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E. M. Sporn gave us helpful assistance in the work.

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TABLE I.
Response in Weight, Hemoglobin and Differential Leucocyte Count of Monkeys Deficient in the Anti-anemia Factor to Milk and Milk Preparations.

| | | Wt (g) | | Hemoglobin (g/100 cc) | | Differential leucocyte count Before | | After 3 wk | |
|---|---|---|---|---|---|---|---|---|---|
| Monkey No. | Treatment | Before | After 3 wk | Before | After 3 wk | % neutrophiles | % lymphocytes | % neutrophiles | % lymphocytes |
| 186 | Raw whole milk | 3140 | 3460 | 12.77 | 13.66 | 63 | 30 | 21 | 62 |
| 217 | ” ” ” | 4000 | 4200 | 12.59 | 14.97 | 59 | 27 | 25 | 67 |
| 82 | Pasteurized whole milk | 3420 | 3620 | 10.43 | 13.33 | 48 | 44 | 26 | 60 |
| 276 | Pasteurized whole milk | 2230 | 2300 | 12.77 | 13.56 | 50 | 37 | 25 | 64 |
| 248 | Raw cream | 2800 | 2910 | 12.60 | 13.11 | 49 | 42 | 39 | 55 |
| 277 | ” skim milk | 2900 | 3240 | 11.61 | 13.71 | 62 | 28 | 30 | 65 |
| 189 | ” whey | 3600 | 3900 | 12.41 | 13.77 | 80 | 20 | 17 | 72 |

ing of a corn grits basal diet.[4] All of these monkeys showed poor weight gains, suboptimal hemoglobin levels, and reversed neutrophile-lymphocyte ratios, the typical syndrome in a deficiency of the monkey anti-anemia factor. If allowed to continue on the assay rations without further treatment, the animals become progressively worse and die within 2 months. The relative potency of the materials to be tested was determined by the response in weight, hemoglobin and differential leucocyte count observed in the monkey during a 3-week period. The milk and milk derivatives were given together with the vitamin supplements each day. In all trials at least one monkey on the corn grits diet and one on the riboflavin diet were used.

*Results.* Seven monkeys were given 50 cc of raw whole milk per day. The animals took this supplement very readily and the response of 2 typical monkeys, No. 186 and 217, is shown in Table I.

Two other monkeys, No. 82 and 276, were given 50 cc of pasteurized whole milk per day, and a definite response was elicited, although it was of a smaller magnitude than the response to raw whole milk (Table I.) This suggests that pasteurization causes some destruction of the monkey anti-anemia factor and verifies the earlier observations as to the lability of this factor.[1,2]

[4] Cooperman, J. M., McCall, K. B., Ruegamer, W. R., and Elvehjem, C. A., *Fed. Proc.*, 1946, **5**, 230.

Since Van Wagtendonk *et al.*[5] reported that raw cream contains a labile anti-stiffness factor essential for guinea pigs, the activity of raw cream for the monkey was determined. Monkeys 248 and 249 were given 10 cc of raw 35% cream. Two other monkeys, No. 277 and 166, were given 50 cc of raw skim milk per day. It is apparent from Table I that the larger portion of the material remained in the skim milk and that all of the activity of the cream could be accounted for by its skim milk content. It was thus evident that the monkey anti-anemia factor is distinct from the guinea pig anti-stiffness factor.

Since raw skim milk was apparently a good source of the monkey anti-anemia factor, further fractionation was attempted. Raw skim milk was treated with a commercial rennet preparation to coagulate the casein and then centrifuged. The clear whey was fed at a level of 50 cc per day to monkey 189. After one week on this regimen the monkey failed to show a significant weight gain. In all probability the casein had carried down some of the activity. However, when the level of whey was raised to 100 cc per day, the monkey started to gain at a rapid rate, as can be seen in Table I. Monkey 186 suddenly started to lose weight. It was then given 100 cc of raw whey per day and the weight loss was checked after one day, and during the next week the monkey gained weight rapidly.

[5] Van Wagtendonk, W. J., Schocken, V., and Wulzen, R., *Arch. Bioch.*, 1944, **3**, 305.

*Summary*. Raw whole milk is a good source of the monkey anti-anemia factor. Pasteurization of the milk seems to reduce the amount of this factor in milk to some extent. This factor can be differentiated from the guinea pig anti-stiffness factor by the fact that raw cream possesses little activity while the raw skim milk accounts for the greater portion of the activity in whole milk. Raw whey, made by enzymatic treatment of raw skim milk, is also a good source of the monkey anti-anemia factor.

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# Effect of Adrenocorticotrophic Hormone on Urinary Nitrogen Excretion in the Normal Rat.*

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It is well recognized that adrenocorticotrophic hormone (ACTH) and adrenal cortical steroids inhibit normal body growth.[1-4] Retardation of chondrogenesis and osteogenesis in the region of the proximal epiphysis of the tibia has likewise been produced by ACTH and has been shown to be mediated by the adrenal.[5] In the hypophysectomized animal, ACTH has been shown to inhibit the growth produced by growth hormone.[6] Since it is generally recognized that nitrogen retention accompanies somatic growth, increased nitrogen excretion might be anticipated following the injection of ACTH. Such a finding is reported in this paper.

Normal, year-old, "plateaued" female rats of the Long-Evans strain weighing between 250 and 320 g were fed 12 g daily of a purified† diet which was completely consumed by all rats. Individual urines were collected at 24-hour intervals. Total urinary nitrogen was determined by the micro-Kjeldahl method. The ACTH was prepared by the procedure described previously.[7,8] Following a control period of 24 days, 5 rats were injected subcutaneously with 1 mg of hormone 6 times daily for 6 days. Six control animals were similarly injected with the same amount of

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† The diet consisted of alcohol-extracted casein 24%, sucrose 63.5%, hydrogenated vegetable oil (Crisco) 8%, salts[11] 4%, and liver fraction powder 0.5%. Crystalline B vitamins are added per kilogram diet; thiamine HCl 5 mg, pyridoxine HCl 5 mg, riboflavin 10 mg, *p*-aminobenzoic acid 10 mg, nicotinic acid 20 mg, calcium pantothenate 50 mg, inositol 400 mg, and choline chloride 1 g. One cc of a fat-soluble vitamin mixture containing 6 mg alpha-tocopherol, 115 chick units vitamin D, 800 U.S.P. units vitamin A, and 650 mg corn oil (Mazola) is given weekly.

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