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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Opposing Effects of ACTH and GH on Body Weight and Urinary Nitrogen Excretion of Pair-Fed Plateaued Female Rats

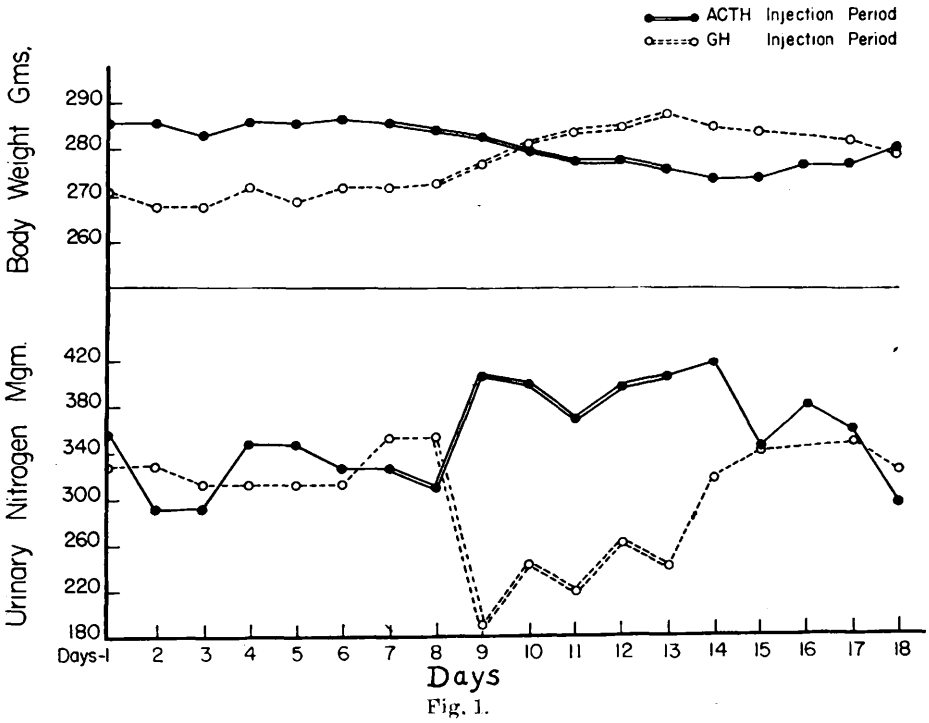

Fig. 1.

protein derived from crude anterior pituitary gonadotrophic extract, inactivated by boiling.

In the 10 days prior to injection, the daily nitrogen excretion per rat was 326 ± 7.8‡ mg and 321 ± 12.4 mg for the experimental and control group respectively. In the first 24 hours of ACTH injection, no change in nitrogen excretion occurred. During the last 5 days of ACTH injection, the daily excretion increased to 394 ± 8.0 mg, an increase of 20.8%, and this increase persisted in the 24-hour period after the injections were stopped. This increase was found to be statistically significant, having a p value of 0.01.[9] Twenty-four hours after stopping ACTH the excretion fell to 343 ± 9 mg, approximately the preinjection level.

In the control group, the daily nitrogen excretion, which had been 321 ± 12.4 mg prior to injection, rose to 352 ± 8.0 mg, an increase of 8.8%, during the injection period. This increase was found not to be highly significant, having a p value of .035.

Decrease in body weight occurred concomitantly and proportionately to the increased nitrogen excretion.§ In the experi-

‡ Mean ± standard error.

[9] Fisher, R. A., *Statistical Methods for Research Works*, 6th ed., Edinburgh, London, Oliver and Boyd, 1936.

§ The urines of experimental animals were also examined for reducing substances by the Somogyi method. Glycosuria developed in but one rat on the first, second, and sixth days of the injection period. It may be recalled that Ingle *et al.*[10] have induced glycosuria in normal rats with ACTH using a diet containing 15 g of available carbohydrate per day. The discrepancy may therefore be attributed to the difference in experimental conditions.

[10] Ingle, D. J., Winter, H. A., Li, C. H., and Evans, H. M., *Science*, 1945, **101**, 671.

mental group, the average weight prior to injection was 286 g. At the end of the injection period, the weight had decreased to 276 g, and continued to fall to 274 g during the subsequent 24-hour period of continued nitrogen excretion. In the control group, on the contrary, the weight remained constant at 283 g.

For contrast, the data are presented in Fig. 1 for this and a similar experiment using purified growth hormone (GH) 0.5 mg intraperitoneally twice daily. On this graph the opposing effects of ACTH and GH on body weight and urinary nitrogen excretion are apparent.

Summary. ACTH causes an increase in urinary nitrogen excretion with proportionate loss of body weight in the normal rat. The effect of subcutaneous ACTH on nitrogen excretion is manifest on the second day and persists 24 hours after injection is stopped.

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Growth of Japanese B Encephalitic Virus in the Yolk of the Developing Egg.

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In an endeavor to produce a chick-embryo vaccine against the Nakayama strain* of Japanese B encephalitic virus, experiments were undertaken to determine how to develop a more potent virus in the egg. Haagen and Crodel[1] reported the cultivation of this virus on the chorio-allantoic membranes of the developing chick, while Smith and Lennette[2] later reported growth on the membranes of 10- to 13-day-old embryonated eggs. The titers in mice seldom were above 10^{-3}. Sabin[3] and his associates in the investigation of vaccine production considered the use of chick-embryo tissues as unsuitable because of the low potency obtained after inoculation either into the yolk or the allantoic fluid. Since they did not mention the titers obtained nor give any details of the egg work, it was thought of interest to investigate further what potency could be developed in the egg by either the allantoic or the yolk sac routes.

Allantoic route. Ten- to 11-day-old embryonated eggs were inoculated with 0.2 cc of a 10% mouse brain suspension through the air sac membrane into the allantoic fluid. To determine the most suitable incubation period for optimum virus growth, eggs were opened in 24, 48, 72, and 96 hours, respectively, after inoculation. Twenty percent suspensions of the embryos and chorio-allantoic membranes in buffered saline were prepared by grinding in a small Waring blendor for 2 minutes. After reduction to a 10% suspension, each material was titrated intracerebrally in mice in 0.03 cc amounts and the 50% endpoint obtained according to the method of Reed and Muench.[4] With the exception of a low titer ($10^{-3.74}$) in 24 hours, the other endpoints remained fairly constant—$10^{-6.2}$ to $10^{-6.5}$ and $10^{-6.74}$. Most of the embryos were alive, except on the fourth day.

Live embryos taken on the third day of incubation were then used for passage material, 16 serial passages being made in this

* The Nakayama strain was kindly sent from the Department of Virus Research at Lederle Laboratories.

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