

Summary. The average urinary excretion of vit. B₁₂ in 14 subjects on normal diets was found to be 31 my per day. Similar values were obtained in 28 subjects maintained for 3 days on the following diets: milk, alkaline ash, low protein, and high protein. A somewhat higher value was found in 8 subjects on high purine diets, and the increase over normal was shown to be due to interfering compounds. On the third day of fasting, 6 subjects were

found to excrete an average of 62 my vit. B₁₂ per day.

The average CF excretion by the subjects on the above diets accounts for only part of the total FA compounds excreted, and appears to be correlated with the total FA values. Differences in vitamin B₁₂ excretion resulting from dietary changes are not similar to those observed in CF and FA excretion.

Received July 23, 1951. P.S.E.B.M., 1951, v77.

Effect of Cortisone upon Chemical Composition of Allantoic Fluid of the Chick Embryo.* (18942)

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The studies herein presented were undertaken in a search for biochemical correlates of the striking changes in the growth, development, and survival of chick embryos treated with cortisone or certain other steroids(1-5).

Methods. Distilled water or dilutions of cortisone† in distilled water were inoculated into the yolk sac of fertile White Leghorn eggs on the fourth day of incubation.

* Aided by a grant from the Damon Runyon Memorial Fund for Cancer Research, Inc.

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2. Karnofsky, D. A., *Fed. Proc.*, 1950, v9, 290.

3. Kilbourne, E. D., and Horsfall, F. L., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1951, v76, 116.

4. Roberts, E., Karnofsky, D. A., and Frankel, S., *Proc. Soc. Exp. Biol. and Med.*, 1951, v76, 289.

5. Karnofsky, D. A., Ridgeway, L. P., and Patterson, P. A., *Endocrinology*, 1951, v48, 596.

† Vehicle in which cortisone acetate is supplied by Merck and Co., Inc:

1.5% benzyl alcohol

0.9% sodium chloride

0.4% polyoxyethylene sorbitan monooleate

0.5% sodium carboxymethylcellulose

(distilled water q.s. ad 100%)

This suspension was diluted with water to permit the injection of 0.25 to 4.0 mg of cortisone in a constant volume of 0.2 ml.

Allantoic fluids for chemical analysis were drawn from surviving embryos on the thirteenth day of incubation. This age was selected to avoid the excessive precipitate of urates in older embryos. The methods employed in the determination of sodium, potassium, chloride and sugar (reducing substance) levels are in regular use in this and in other laboratories(6).

Results. In Fig. 1 the chemical composition of the allantoic fluid from cortisone-treated chick embryos has been compared with the findings in controls inoculated with distilled water.

It is readily evident that cortisone treatment raised the mean value of the allantoic fluid sodium and lowered its potassium. This is a consistent finding irrespective of the amounts of hormone given. There is no dosage-effect relationship present despite the fact that the graphs suggest maximal increases in sodium in the 1.0 mg group with alterations in the same direction but of lesser magnitude with the 0.25, 0.5, 2.0, and 4.0 mg amounts. Statistically significant changes in the mean chloride and sugar (reducing substances) were

6. Danowski, T. S., Peters, J. H., Rathbun, J. C., Quashnock, J. M., and Greenman, L., *J. Clin. Invest.*, 1949, v28, 1.

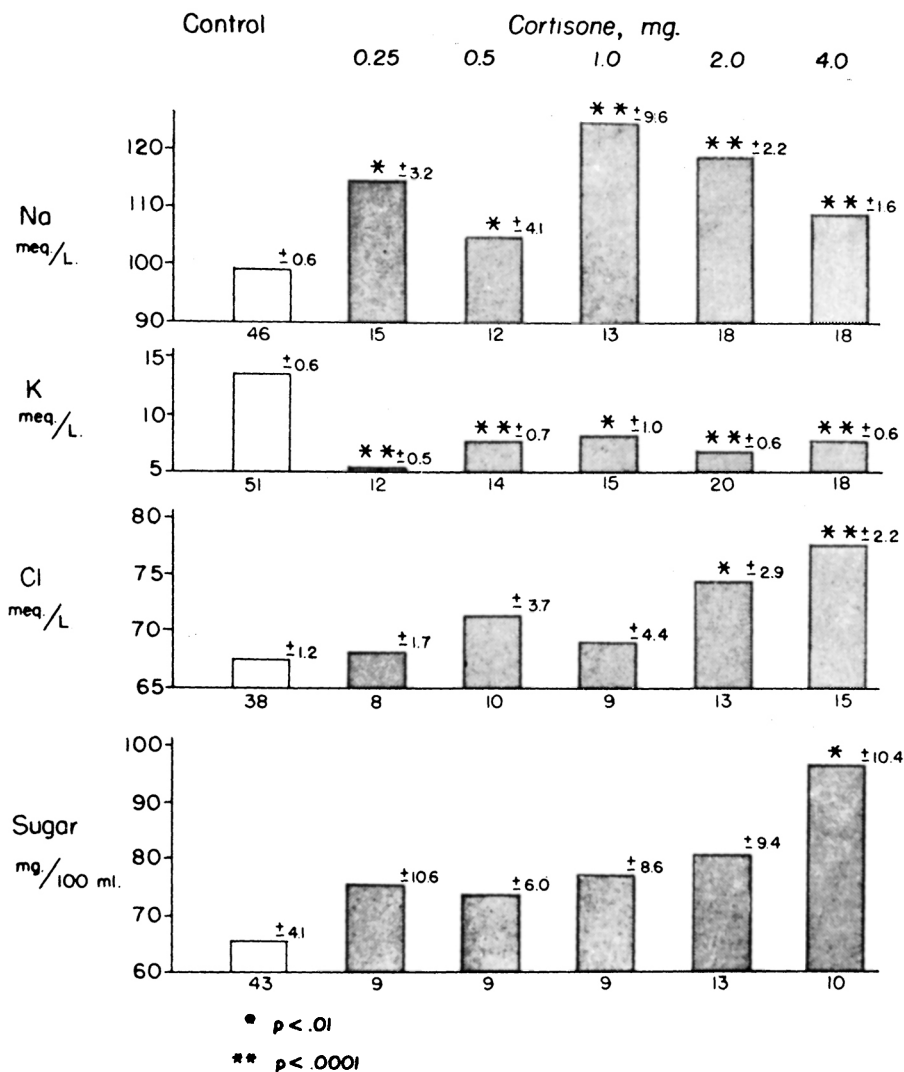


FIG. 1. Mean concentrations ($\pm$ S.E.) of ions and reducing substances in allantoic fluids of 13-day-old control or cortisone-treated chick embryos. Injection of cortisone, 0.25 to 4.0 mg, into the yolk sac of 4-day-old fertile eggs increased the mean concentration of sodium and lowered the potassium. The chloride values rose in the 2.0 and 4.0 mg groups, while the reducing substances increased significantly in only the latter.

recorded only with the 2 larger and the largest dosages of cortisone, respectively. The findings are not attributable to changes in hydration, since the weights of the control and the treated eggs were indistinguishable at the end of the experiment. The opposing directions of the sodium and potassium alterations exclude the possibility that the observed changes merely indicate a decrease in the water of allantoic fluid.

Discussion. The allantoic fluid has been

taken to represent the main excretory pool in the chick embryo(7,8). As such the effects of cortisone in these studies can be compared for purposes of discussion with those reported in the urine of human subjects receiving this steroid. The sodium and potassium effects are just the opposite of the changes in urine

7. Needham, J., *Chemical Embryology*, University Press, Cambridge, England, 1931.

8. Patten, B. M., *Early Embryology of the Chick*. The Blakiston Co., 4th edit., Philadelphia, 1951.

composition generally found to follow the initiation of cortisone therapy in human subjects(9), *i.e.*, the excretion of sodium into the allantoic fluid increased while the output of potassium decreased. These findings suggest that the chick embryo allantois is influenced by cortisone, directly or indirectly, but responds in a manner opposite to that characteristic of the human kidney. The increased concentration of chloride and of reducing substances in the allantoic fluid, on the other hand, is directionally similar to the changes induced by cortisone in humans.

It may be that exogenous cortisone interferes with the formation or function of the adrenal gland and thereby ultimately induces adrenal insufficiency. In such an event the observed cation and chloride changes would resemble those seen in human adrenal insufficiency(10). Since the unhatched chick

lives and grows in a closed system, any increased output of sodium or decreased elimination of potassium must inevitably be accompanied by depletion of the former element from the body and excessive retention of the latter. Our data do not indicate whether the chemical changes mediate or merely accompany the retardation of growth, developmental anomalies, and decreased survival rates observed in cortisone-treated chick embryos.

Summary. 1. The observed rise in sodium and the decrease in potassium in the allantoic fluid of chick embryos treated with cortisone are the reverse of the reported changes in the urine of human subjects receiving this steroid, and resemble those seen in adrenal insufficiency. 2. The increased chloride and sugar levels in the fluid of the allantois following therapy with the higher dosages of cortisone resemble directionally the renal effects of cortisone in man.

9. Ingle, D. J., *J. Clin. Endocrinol.*, 1950, v10, 1312.

10. Danowski, T. S., *Am. J. Med.*, 1951, v10, 468.

Received July 23, 1951.

P.S.E.B.M., 1951, v77.

Inhibition of the Growth of Egg Cultivated Tumor Tissue by Extracts of *Cooperia pedunculata* Herb. (18943)

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During a survey of the effect on tumor tissue of various extracts of plants known to be toxic when ingested by animals, certain extracts of *Cooperia pedunculata** Herb. (Rain-lily) have proved to be inhibitory of tumor growth.

Acetone and acid aqueous extracts of the bulb of this plant when introduced into eggs bearing yolk sac implantations of mouse mammary carcinomas inhibited the growth of the tumor tissue almost entirely for the period of the test at dosage levels which did not appear to affect adversely the supporting chick embryos.

The plant *Zephyranthes atamasco* Herb. is known to be poisonous to live stock(1). The Rain-lily used in this study is a closely related species and probably has the same toxic properties.

No reports were found in the literature of the use of Rain-lily extracts in cancer chemotherapy.

Included in this report are results obtained when 4-aminopteroylglutamic acid (Aminopterin) was tested in the same way as the plant extracts. This compound is well known for its inhibitory effect on the growth of cancer and other tissues and the fact that this is readily demonstrated by injection into tumor

* Our appreciation is extended to Dr. W. V. Brown and Dr. G. S. Rabideau of the Department of Botany, University of Texas, for confirming our identification of this plant.

1. West, Erdman, and Emmel, M. W., Bull. No. 468, Agr. Exp. Sta., University of Florida, 1950.