Influence of Cortisone on Free Hydroxyproline in the Developing Chick Embryo. (18466)

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In the course of the chromatographic examination of the free amino acids and peptides of the tissues of the developing chick embryo, determinations were also made on extracts prepared from tissues of embryos which had received single injections of 1 mg of cortisone (cortisone acetate-Merck) onto the chorioallantoic membrane. The cortisone-treated embryos were tested because of the marked and specific inhibition in their growth produced by the hormone(1). The most consistent difference observed between the tissues of the normal and cortisone-injected embryos was a large increase in the content of free hydroxyproline in the latter. It is the purpose of this communication to report some of the details of this finding.

Experimental. Embryos and preparation of tissues. The tissues were obtained from 3 batches of fertile eggs. The first 2 groups were from a sex-linked Barred Rock stock obtained during the summer of 1950 in Bar Harbor, and the third was from White Leghorns obtained in New York City in the fall of the same year. The eggs were incubated at 38°C and 75% relative humidity and were kept in one position, being moved once daily when candled for viability. The injections of cortisone were made onto the chorioallantoic membrane in the manner previously described(1). The injections were made on the eighth day, since the cortisone-effect appears to be initiated between the eighth and tenth days, even when the hormone is given earlier(1). In the third set of eggs a control group was also injected with Δ^1-17 α-hydroxy progesterone, a steroid not possessing any cortisone-like activity. In the first and third set of eggs the embryos were sacrificed at 18 days of age and then weighed and examined grossly for the typical effects produced by cortisone(1). In the second group the surviving embryos were sacrificed at various earlier times as well as at 18 days. The effect of the cortisone on growth and feather formation was considerably less in the Barred Rock embryos than in the White Leghorns. The growth rate of the normal embryos of the latter strain was also generally slower. Extracts of the freshly excised tissues were prepared for analysis essentially as previously described(2) with the exception that the salt was removed from a number of the samples by electrolytic desalting(3).

Chromatographic and chemical procedures. Aliquots corresponding to 75 mg of fresh tissue were placed on paper for 2-dimensional paper chromatography by the conventional methods(4,5). In several instances larger aliquots were employed to detect substances present only in small amounts. Care was taken to run the extracts of the comparable control and cortisone-treated embryos through the entire preparative and chromatographic procedure at the same time, so that slight

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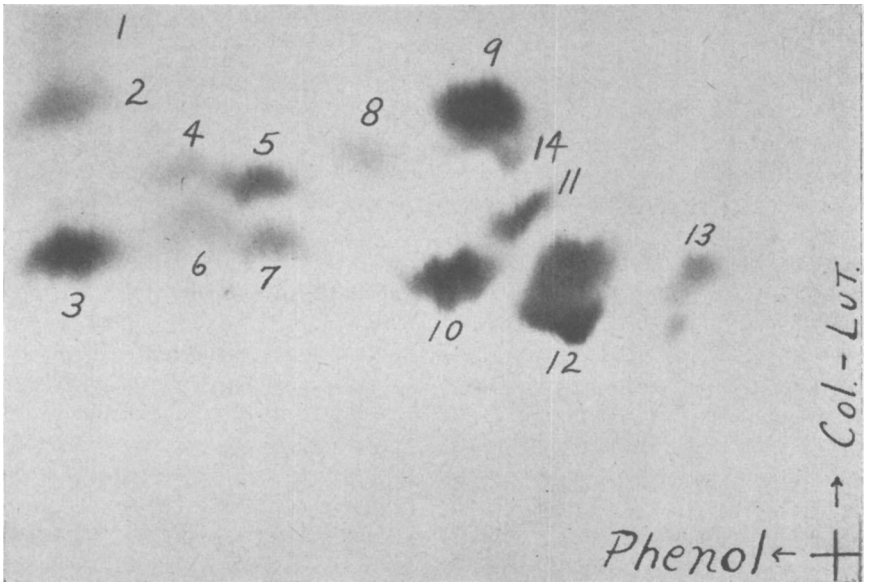

FIG. 1

Chromatogram of extract of 150 mg of brain of 18-day cortisone-injected chick embryo. Key to numbers on chromatograms: Leucines, 1; valine, 2; γ-aminobutyric acid, 3; hydroxyproline, 4; alanine, 5; β-alanine and/or citrulline, 6; glutamine, 7; threonine, 8; taurine, 9; glycine, 10; serine, 11; glutamic acid, 12; aspartic acid, 13; unknown substance, 14.

variations in conditions would not influence the results. In some instances it was possible to analyze the extracts for hydroxyproline content by a recently devised colorimetric procedure(6).

Chromatographic results. In all cases chromatograms of the extracts of brains, livers, and hearts of 14, 16, and 18-day embryos showed notable increases in the content of free hydroxyproline in the cortisone-injected animals within each experimental set as compared with the same tissue of the comparable control. The identity of the hydroxyproline was established by the characteristic orange-brown color, the position on the chromatogram, and the complete superimposition of the spots formed when pure hydroxyproline was added to the tissue extracts. In a number of cases, but not in all, the tissues of the cortisone-treated chicks also seemed to contain somewhat more free glycine than the controls. There were no consistently observable differences in the concentrations of the other ninhydrin-reactive constituents. In the tissues taken from 9 through 12 days the patterns of free amino acids were virtually identical in the normal and injected embryos.

In Fig. 1 and 2 are shown chromatograms from comparable aliquots of desalted extracts of brain from pooled samples of 18-day embryos with and without cortisone treatment. Photographs in Fig. 1 and 2 were taken with a blue filter in order to accentuate the color due to hydroxyproline. The spot produced by hydroxyproline (spot 4) was too light in the case of the control tissue to be reproduced on the photograph. It is obvious that the content of this amino acid is higher in the cortisone-injected embryos. In most cases the chromatograms were virtually indistinguishable except for the intensity of spot 4 and sometimes spot 10 (glycine). Although occasionally some of the other ninhydrin-reactive constituents were present in somewhat larger amounts in the tissues of either the normal or cortisone-treated embryos, these were not consistent observations. The original chromatograms were shown in pairs to several individuals not di-

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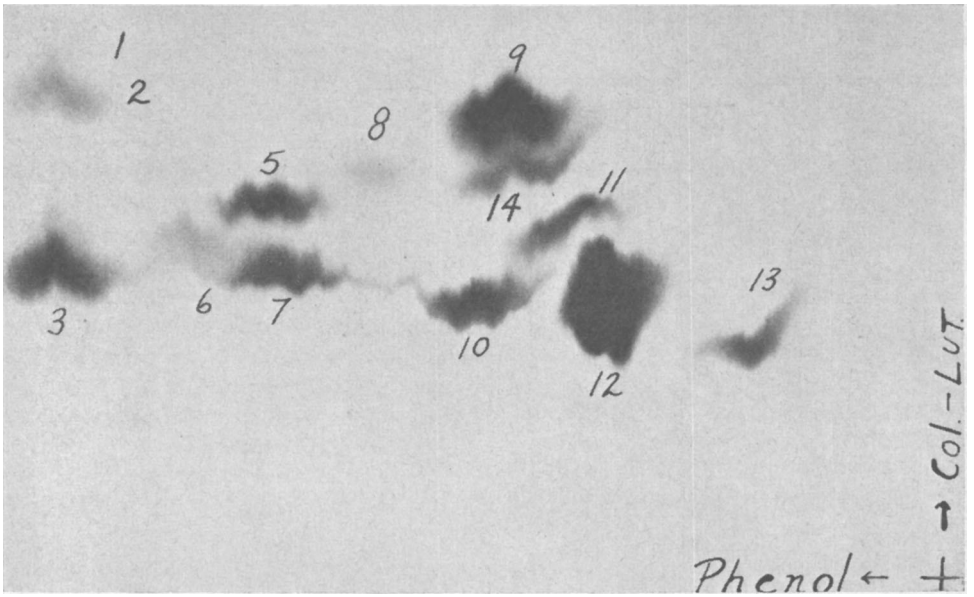

FIG. 2.
Chromatogram of extract of 150 mg of brain of 18-day control chick embryo.

rectly concerned with the experiments, and they were asked to separate them into two groups on the basis of the intensity of the hydroxyproline spot. Employing this criterion within each experimental group they were able to separate all of the chromatograms from the tissues of the cortisone-treated embryos from those of the same tissue of the controls of the same age without a single error.

Colorimetric determination of hydroxyproline content. While the present experiments were in progress a colorimetric procedure for the determination of hydroxyproline became available(6). Sufficient quantities of some of the frozen extracts were left to enable us to perform the analyses shown in Table I. It is seen that in each series the tissues of the cortisone-treated embryos contained more hydroxyproline than did those of the comparable controls. The effect was most marked in the brain. Δ^1-17 α-hydroxy progesterone seemed to depress the level of hydroxyproline slightly. The embryos of the second series showed less effect of the cortisone than did the other two groups as judged by the inhibition of growth and feather formation. In this group the levels of free hydroxyproline were lower both in the controls and in the cortisone-treated embryos than in the third series. However, the *relative* increases in hydroxyproline were approximately the same in both groups.

Discussion. Hydroxyproline was not detected in 5 purified egg proteins(7) and was also absent from the egg white and yolk of the unincubated egg(8). It has been shown to accumulate in the embryo and embryonic membranes as development proceeds, the first traces of this amino acid becoming detectable in the embryo after the fourth day(8). The results of the latter investigator also suggest that the hydroxyproline of the embryo is present principally in the form of collagen or collagen-like substances. The present investigation shows that the only consistent abnormality in the pattern of free amino acids of the tissues of the developing chick embryo produced by the injection of cortisone was a marked increase in the content of free hydroxyproline. This suggests the possibility that there may be a specific effect of cortisone on the metabolism of hydroxyproline. On the other hand, this increase in free hydroxy-

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TABLE I. Effect of Cortisone on Content of Free Hydroxyproline in the Tissues of 18-Day Chick Embryos.

Series	Treatment	Hydroxyproline content; γ per 100 mg of fresh tissue		
		Brain	Liver	Heart
1	Control	12.0	—	—
	Cortisone	68.0	—	—
2	Control	4.0	3.8	5.6
	Cortisone	15.9	9.8	11.3
3	Control	12.0	10.0	12.4
	Inactive steroid*	10.4	6.4	8.4
	Cortisone	30.2	26.4	32.8

* Δ^1-17 α-hydroxy progesterone.

proline may only indirectly reflect the inhibition of the synthesis of collagen or collagen-precursors.

Summary. A chromatographic examination was made of the free amino acids of tissues of normal and cortisone-injected chick embryos. The content of free hydroxyproline was increased markedly in the tissues of the cortisone-treated embryos. The results were confirmed by a colorimetric procedure. With the exception of glycine, in which increases were noted in some instances, the other amino acids showed no consistent changes as a result of the injection of cortisone. The injection of Δ^1-17 α-hydroxy progesterone produced slight decreases in free hydroxyproline content.

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Absorption and Excretion of Viomycin in Humans.* (18467)

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Viomycin, a derivative of a species of *Streptomyces,* is a compound which has been found to have antimicrobial activity for a number of species of bacteria *in vitro* and to protect mice against experimental infections (1,2). It has been of particular interest because of an inhibitory effect upon mycobacteria, including *M. tuberculosis,* and because of its ability to suppress tuberculosis in animals(3). Preliminary clinical studies with viomycin in tuberculous patients have been carried out(4). The present study concerns the absorption and excretion of viomycin in human subjects. In addition, serum concentrations in patients receiving maintenance therapy and concentrations in the cerebrospinal fluid have been determined.

Materials and methods. Preparation of drug. Viomycin‡ was supplied as the highly purified sulfate salt in 1.0 g vials and was dissolved in sterile distilled water in a concentration of 250 to 500 mg per ml for intramuscular injection.

Collection of specimens. In the studies of absorption and excretion after a single intramuscular dose, specimens of blood were drawn under sterile precautions at prescribed inter-

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‡ Supplied by Charles Pfizer and Co., Brooklyn, N. Y.