XX. CHOLINE AS A PRECURSOR OF GUANIDINE. THE DECREASE IN THE AMOUNT OF CHOLINE OF THE HEN'S EGG DURING INCUBATION.

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GUANIDINE not only occurs in the protein molecule as arginine but it is also found in muscle in simpler combination as creatine—methylguanidineacetic acid—and also as methylguanidine [Smorodinzew, 1923].

In tetania parathyreopriva Burns and Sharpe [1917] found the amount of methylguanidine markedly increased in the blood and urine and in idiopathic tetany, in the urine.

The source of guanidine in the body is still unknown. Burns [1916] showed that its amount increases in the hen's egg during incubation, but no evidence of its source was adduced.

Riesser [1913, 1914] suggested the possible formation of methylguanidine-acetic acid from choline by union with urea according to the equations:

By oxidation of the acid, methylguanidine would be yielded.

He adduced evidence that the creatine of muscle is increased after the administration of choline.

Baumann, Hines and Marker [1916] state that on perfusing with choline and urea they found an increase of creatine in the muscles of the dog. Shanks [1921] found that the intravenous administration of choline is followed by a small increase in the creatine of muscle.

Choline occurs in the lecithin molecule in the hen's egg. If it is a precursor of guanidine a decrease in its amount in the egg should occur during incubation while the guanidine is increasing.

METHOD.

The eggs were weighed and the contents of each ground up with sand. This was then repeatedly extracted with alcohol containing about 10 % of ether. The extract after filtration was made to a definite volume and one-tenth

taken for analysis. Hydrolysis of this portion of extract with excess of $\text{Ba}(\text{OH})_2$ in 50 cc. of water was then carried out, boiling under reflux condenser for one hour and filtering while hot. 5 % sulphuric acid solution was then added to precipitate the excess of barium and any excess of the acid was neutralised by the addition of cream of BaCO_3 . The fluid was then filtered. This filtrate should be perfectly clear. It was then evaporated almost to dryness, dissolved in about 10 cc. of distilled water and filtered. Iodine potassium iodide solution was now added and the estimation of the choline completed as described in a previous publication [Sharpe, 1923].

Six eggs were separately analysed at each period; (i) unincubated, (ii) 7th day, (iii) 14th day and (iv) 21st day of incubation. In some cases the analyses were not completed, but the correspondence between the results was so close that averages for the series analysed at each period are given. They show a steady decrease in the choline content.

RESULTS.

Amounts of choline in the egg of the fowl at different periods of incubation.

Day	Total in g.	%
Unincubated	0.24	0.40
7th	0.21	0.38
14th	0.19	0.32
21st	0.11	0.22

Taking Burns' curve for his sets II to V and expressing it as guanidine it is seen that the rise in the amount of guanidine per egg is from 0.08 g. to 0.26 g., *i.e.* 0.18 g. The decrease in choline found in the present series was from 0.24 g. to 0.11 g., *i.e.* 0.13 g. According to Riesser's equation, 1 g. of choline should yield about 2 g. of guanidine.

These results strongly support the view that choline is a source of guanidine in the animal body.

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