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Changes in the Form of Inositol During Incubation of Eggs.

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Snell and Quarles[1] have reported recently that the content of inositol in eggs increases about 6 times during the period of incubation. Since the embryo was apparently able to synthesize this vitamin, it was suggested that the more mature organism was also possessed of this power, and hence that inositol was not a dietary essential for chicks. Analyses were performed on aqueous extracts of embryos which had been allowed to autolyze for short periods. Recently it has been demonstrated that at least 4 forms of inositol occur in tissues, *viz.*, free inositol, inositol hexaphosphate,[2] a new phosphatide,[3, 4] and a water-soluble, non-dialyzable substance.[5, 6] It has also been found that yeast responds only to free inositol.[7] Since several of the combined forms of inositol are not split by autolysis and since the response of yeast was the basis of the method of analysis used by Snell and Quarles, it seemed possible that what they had observed was a conversion of combined inositol to the free substance during incubation of eggs. It was, therefore, of interest to reinvestigate the problem with the aid of an analytical method which determines total inositol as well as, but separate from, free inositol.[6, 8] Total inositol was estimated by the method of Woolley[8] and free inositol by the modification recently described.[6] Fertile eggs were obtained from commercial sources (not always the same). They were incubated at 38.9°C and 70% humidity in a standard incubator. All analyses were based on the weights of the eggs at the beginning of the period of incubation. Before an egg was taken for analysis it was candled in order to assure that only living embryos were examined. Three separate experiments were performed. In the first, groups of 2 eggs were analyzed for free and total inositol at 0 and 20 days.

* With the technical assistance of A. G. C. White.

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6 Woolley, D. W., *J. Exp. Med.*, 1942, **75**, 277.

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TABLE I.
Average Inositol Content of Eggs at Various Stages of Embryonic Development. Numbers in parentheses indicate maximal deviation from the mean.

Incubation, days	No. of eggs	Avg inositol content, μg/mg	
		Total	Free
0	11	.22 (± .05)	
	18		.046 (± .01)
7	2	.24 (± .0)	
10	2	.21 (± .04)	
14	2	.26 (± .04)	
20	10	.24 (± .03)	
	8		.19 (± .05)

and for total inositol at 7 and 14 days of incubation. In the second, the eggs were taken after 0, 10, and 20 days of incubation. In the third, groups of 6 eggs were analyzed at 0 and 20 days of incubation.

The average content of inositol in an egg at various periods of incubation is shown in Table I. The values for free inositol at the beginning and end of the incubation period are also shown. It can be seen that the total inositol in an egg did not increase significantly during the period of embryonic development. However, the proportion which is free increased. All of the inositol in aqueous extracts of eggs before incubation was free, and hence it was concluded that the combined inositol in such eggs was present as one of the water-insoluble forms. The fact that nearly all of the inositol in a hatching chick is free is of interest when it is recalled that in many tissues only about half of the vitamin is in the free state.[6]

Summary. The total content of inositol in a hen's egg does not increase during incubation, but the proportion of the total amount which is free and extractable by water does increase.

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Glycine Requirement of the Chick.

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It has been shown that glycine is required in the diet of the young chick for an optimum rate of growth.[1, 2, 3] Experiments have been

[1] Almquist, H. J., Stokstad, E. L. R., Mecchi, E., and Manning, P. D. V., *J. Biol. Chem.*, 1940, **134**, 213.