XXVII. A NOTE ON THE PRESENCE OF VITAMIN C IN THE CHICK EMBRYO.

By SURENDRA NATH RAY.

From the Biochemical and Nutritional Laboratories, University of Cambridge.

(Received December 23rd, 1933.)

It has long been known that fowls and other birds resemble rats [Parsons, 1920] and dogs [Harris, 1931] in having little apparent need for vitamin C in their diet. Plimmer *et al.* [1923] concluded that the growth of the chick in the egg, which is known to be free from vitamin C, is one of the best indications that vitamin C is not needed by the growing bird. This statement has been criticised on the ground that the avian embryos may synthesise the vitamin during their development, and indeed Carrick and Hauge [1925] and Hart *et al.* [1925] demonstrated the presence of vitamin C in the livers of chicks which had been fed for a long time on vitamin C-free diets. Hauge and Carrick [1926] further showed that though both the yolks and the whites of eggs laid by hens which had been on scorbutic ration contained no demonstrable amount of vitamin C, yet the livers of five-weeks-old chicks hatched from these eggs and fed on scorbutic rations contained an abundance of this vitamin. This makes it evident that during incubation vitamin C is being produced in the growing embryo.

The earlier studies, which involved the employment of large amounts of embryonic tissue for biological assay, prevented the actual detection of vitamin C in the growing embryo. The much simpler micro-chemical method of Birch *et al.* [1933] makes the demonstration of the vitamin an easy and rapid affair.

EXPERIMENTAL.

Brown Leghorn hens' eggs were used. The eggs after the proper time of incubation were opened, the embryos removed and dried on filter-paper. They were rapidly weighed and ground up with a little sand and sufficient 20 % solution of trichloroacetic acid to give a final concentration of the latter of about

Table I.

Day of incubation	Weight of the substance taken g.	Total amount of ascorbic acid mg.	Amount of ascorbic acid in mg./g. wet tissue	Amount of ascorbic acid in mg./g. dry tissue
0	(a) Egg-white 27	0	0	0
	(b) Egg-yolk 20	0	0	0
4	(a) 5 whole embryos 0.6	0.0745	0.124	2.98
	(b) Egg-white 11.6	0	0	0
	(c) Egg-yolk 20	0	0	0
6	2 whole embryos 0.89	0.143	0.160	3.04
8	1 " 1.17	0.213	0.182	2.91
10	1 " 2.68	0.630	0.235	3.38
12	1 " 5.05	1.260	0.249	2.81
15	Embryo livers 0.50	0.132	0.264	—
17	" 0.37	0.105	0.283	—
19	" 0.65	0.178	0.274	—
21	" 0.82	0.226	0.275	—
24 hours old chick-liver	1.05	0.273	0.260	—

5 %. The extract was then made up to a suitable volume and the filtrate titrated against a measured volume of a standard solution of 2 : 6-dichlorophenolindophenol. Up to the 15th day the whole embryos were used; on the 15th day and after, the livers only were examined.

The dry weight of the embryo, used to calculate the figures in the last column of the table, was obtained from Murray's [1926] figures for chick embryo. As the wet weights were found to be nearly identical with his values of wet weight of the embryo, it may be taken that the figures in the last column are reasonably accurate.

DISCUSSION.

It is evident from Table I that while there is no reducing substance in the fresh hens' eggs, quite large amounts of such substances appear in the embryo on incubation. It is also known from previous work [Birch *et al.* 1933; Harris, 1933] that only a few substances other than ascorbic acid reduce the dye under the conditions of the experiment. As these other substances are not likely to be present in the fresh embryo tissue, the amount of reduction can be taken to be a fair indication of the amount of vitamin C present. Further, Hart *et al.* [1925] found that 3 g. of liver from a chicken kept on scorbutic ration for 83 days, constitute a curative dose for scorbutic guinea-pigs; while 1 g. of such liver is definitely not sufficient. If we now take 0.9 mg. of ascorbic acid as the minimum curative dose [Harris and Ray, 1933, 1, 2] and take from Table I the average amount present in liver to be 0.26 mg., the minimum curative dose for chicken liver works out to be 3.4 g. This value is in close agreement with the figures given by Hart *et al.* [1925].

The appearance of vitamin C with the commencement of growth is also seen in the germination of seeds. Moreover, it may be seen from Table I that the amount of vitamin C present per g. of the dry embryo tissue is fairly constant. These facts naturally suggest that there may be some connection between the two phenomena—the appearance of vitamin C and the beginning of growth. In order to determine whether excess of vitamin C has any influence on the rate of growth, some preliminary experiments were performed by injecting about 2 mg. of ascorbic acid (ten times the amount present in the developing embryo) in sterile aqueous solution into the air-space of the eggs before incubation. The result was however totally negative. From both the untreated and treated eggs, the chickens came out simultaneously after the usual incubation period of 21 days, nor was there any great difference between the weights of the two classes. It does not seem likely therefore that moderate excesses of vitamin C have any further direct action on the growth.

SUMMARY.

1. The embryo of the chick has been examined for vitamin C. There is no vitamin present in the fresh egg, though quite a large amount is present even after only 4 days' incubation. The amount of vitamin C present in embryo chick liver agrees very well with the value given by Hart *et al.* [1925] for livers of chickens kept for a long time on a scorbutic ration.

2. Aqueous solutions of vitamin C when injected into the hen's egg do not seem to produce any change in the growth-rate of the embryo.

My thanks are due to Sir F. G. Hopkins for his kind interest in this work. I am indebted also to Dr L. J. Harris and Dr J. Needham for their constant advice and encouragement.

REFERENCES.

- Birch, Harris and Ray (1933). *Biochem. J.* **27**, 590.
Carrick and Hauge (1925). *J. Biol. Chem.* **63**, 115.
Harris (1931). Quoted by Innes, *Ann. Rep. Inst. Animal Path.*
Cambridge (1931), p. 150.
—— (1933). *Nature*, **132**, 27.
Harris and Ray (1933,1). *Biochem. J.* **27**, 580.
—— — (1933, 2). *Biochem. J.* **27**, 2016.
Hart, Steenbock, Lepkovsky and Halpin (1925). *J. Biol. Chem.* **66**, 813.
Hauge and Carrick (1926). *Poultry Sci.* **5**, 166.
Murray (1926). *J. Gen. Physiol.* **9**, 39.
Parsons (1920). *J. Biol. Chem.* **44**, 587.
Plimmer, Rosedale and Raymond (1923). *Biochem. J.* **17**, 787.