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The Order of Utilization of Phosphorus Compounds in the Egg by the Chick Embryo[1]

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In the course of some experiments in these laboratories on autoradiography with P^{32}, we observed that early chick embryos invariably showed a higher deposition of P^{32} than later ones. The result could indicate that the embryo first utilizes the inorganic phosphorus available in the egg for building the phosphorus compounds in its tissues, for we injected P^{32} as NaH_2PO_4. To check on this hypothesis we gathered data on the specific activity of various phosphorus fractions in the embryo.

TABLE 1

SPECIFIC ACTIVITY*

	Average acid soluble	Residual phosphorus	Phosphatide
2-day embryo	10.71	20.3	1.67
4-day "	5.45	8.94	0.50
6-day "	2.23	0.59	0.59

* Specific Activity $= \frac{\text{Counts/}\mu\text{g of phosphorus}}{\text{Counts/ml of original } NaH_2PO_4 \text{ sol}} \times 10^4$

New Hampshire brown eggs from the University of Maryland farm were set aside in three groups to be incubated in a modified Buckeye incubator for 2-, 4-, and 6-day periods. Each of these eggs had received 0.1 ml of an isotonic solution of NaH_2PO_4 with a activity of 0.2 μc. The injections were made from a 1-cc tuberculin syringe with a ½-in. No. 27 needle through a small hole, drilled in the blunt end of the egg into the air chamber while the egg was supported in a cotton-filled cup. We used a No. 54 drill in a small table drill press controlled by a foot pedal. The eggs were sealed with sterile paraffin. The isotonic NaH_2PO_4 was prepared from radioactive KH_2PO_4, procured from Oak Ridge, following standard procedure. The radioactivity of the solution was determined by comparison with Bureau of Standards Ra D+E standard, No. 26. All radioactivity measurements were made with a mica end-window Geiger-Müller tube, window thickness 3.0 mg/cm², feeding into a Tracerlab Autoscaler. The experimental technique was essentially that reported by Branson *et al.* (*1*).

At the end of each incubation period the embryos were removed, frozen immediately in liquid air, and ground to a powder. The powder was extracted several times with cold 10% trichloracetic acid, followed by extraction with cold 5% acid. Separation of the acid solubles into their component parts was not carried out. However, the residue from the acid solubles was extracted with alcohol and ether to remove nucleoproteins and phosphatides. The scheme of separation was based on that of Hevesy *et al.* (*2*).

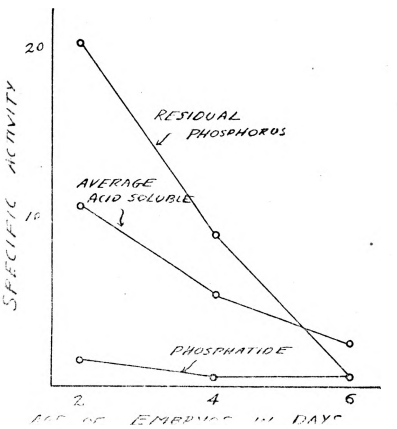

FIG. 1. Behavior of the specific activities for 3 phosphorus fractions.

The phosphorus was determined spectrochemically according to the procedure of Kitson and Mellon (*4*). The results are given in Table 1 and Fig. 1. The values in Table 1 are the averages of 15 2-day old embryos, 11 4-day old embryos, and 11 6-day old embryos, respectively. The embryos were pooled prior to chemical extraction to insure sufficient material for proper handling.

The eggs to be used for autoradiography were prepared in the same manner. The viable embryos were dropped immediately into liquid air. They were allowed to thaw, and the material was prepared following method C of Holt *et al.* (*3*). Ten micron sections were used on Agfa Triple-S Pan film. The sections were given a thin coat of 1% collodion to avoid sticking and the production of artifacts.

Fig. 1 shows that the specific activities of the 3 phosphorus fractions decrease rapidly as the embryo ages. This behavior can be understood only on one tenable hypothesis: the embryo first uses the small amount of

[1] This work has been assisted by the Office of Naval Research, and Research Corporation of New York.

inorganic phosphorus available in the egg to build the phosphorus compounds. (The alternative possibility that the embryo selectively absorbs the P^{32} over the P^{31} requires a renunciation of the isotope tracer technique.) The hypothesis is consistent with earlier experiments in our laboratory and the pioneer work of Hevesy *et al.* (*2*).

The data enable us to make a more specific suggestion as to the mechanism operating in this process. Hevesy *et al.* (*2*) injected hexosemonophosphate into the egg. They found that all the phosphorus fractions in the 14-day embryo were labeled, but that only the inorganic phosphorus and the hexosemonophosphate of the yolk were labeled. We believe that these results and our findings indicate the operation of the following mechanism:

The organic phosphorus compounds are hydrolyzed outside the embryo (possibly by a phosphatase released by the embryo or by the activation of a phosphatase by the embryo), and the inorganic phosphorus produced is then taken up by the embryo. Otherwise, our data would require that both the organic phosphorus and the inorganic phosphorus enter the embryo, since we have repeated the experiment proving that inorganic phosphorus does not label the organic phosphorus when radioactive inorganic phosphorus is stirred with the yolk and permitted to stand at room temperature for several hours. This experiment shows that there is in the yolk no equilibrium of the type: organic phosphorus $\leftrightarrows$ inorganic phosphorus. In addition, the larger amount of phosphorus entering the embryo must be from the inorganic fraction, since the specific activities of all fractions in the 2-day embryo are roughly 4 times those in the 6-day embryo. Kugler showed that, of the 64.6 mg of lipoid phosphorus in the yolk, only 25.1 mg remained there on the 20th day of incubation. Only 7.87 mg of lipoid phosphorus was in the embryo. Thus 31.63 mg had been hydrolyzed to yield inorganic phosphorus. These findings under an alternative mechanism would require the unnecessary transport of 31.63 mg of lipoid phosphorus into the embryo, with the subsequent release of a considerable fraction of the inorganic phosphorus back into the yolk.

This proposed mechanism is consistent with all the information and does not necessitate the assumption that relatively large amounts of inorganic phosphorus must leave the embryo. Although these initial experimental results are not conclusive, they do support the proposed scheme. Whether the embryo does use the organic phosphorus directly has not been determined. It looks as if that point may be difficult to establish. Additional experiments to check further on the proposed mechanism are in progress.

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