plants and bacteria, in all of which manifest changes in fully viable individuals are rather exceptional.

Radiation effects in nematodes have been studied before, but the emphasis has not been on the characters of the adult individuals, as in our investigation. Alicata and Burr (*1*) tried to kill larvae of *Trichinella spiralis* by irradiation with radiocobalt. They found that a dose of 2,000 r per day was not lethal. The eggs of *Parascaris equorum* have been the object of numerous investigations with irradiation, as in the work of Zuppinger (*2*). However, the effects studied were mainly killing and delay of mitosis. As far as we know, changes in the adults like the ones we observed have not been previously described.

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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 an 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*).

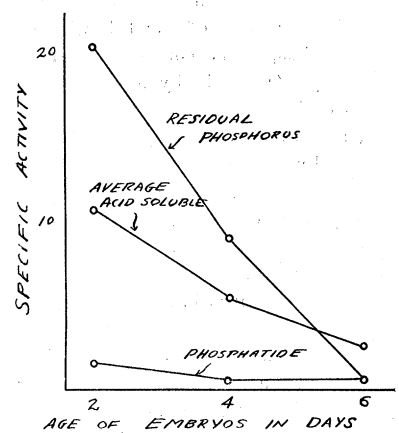

FIG. 1. Behavior of the specific activities for 3 phosphorus fractions.

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*).

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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A Paper Chromatographic Study of Ferritin and Apoferritin Hydrolysates[1]

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During the course of experimentation on some chemical properties of the iron-protein, ferritin, and the role of ferritin in iron absorption, the amino acid composition of ferritin and of its protein moiety apoferritin was studied. Conflicting data concerning the amino acid components of ferritin and apoferritin were reported by Kuhn *et al.* (*4*) and Tria (*6*). Recently, however, Mazur and Shorr (*5*) reported the partial amino acid composition of horse spleen ferritin and apoferritin, which showed identical amino acid values on the basis of nitrogen content.

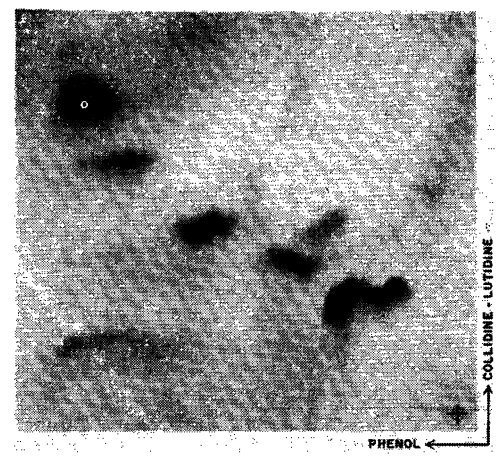

FIG. 1. Paper chromatogram of an apoferritin hydrolysate.

The two-dimensional chromatographic method used in these studies was essentially that of Consden, Gordon, and Martin (*1*) as modified by Dent (*2*). The solvents employed were phenol, saturated with water, and a mixture of 2,4,6-collidine and 2,4-lutidine, saturated with water. Whatman No. 1 filter paper was used. The amino acids were detected by spraying the papers with 0.1% ninhydrin in *n*-butanol to which an excess of water had been added. The color was allowed to develop for 24 hr, and the amino acids were identified by their relative positions and by comparison with a reference map prepared by Dent (*3*).

The chromatogram thus obtained with 50 μl of horse

[1] Material used in this study was taken from a thesis submitted by B. W. G. to the University of Rochester in partial fulfilment of the requirements for the Ph.D. degree in biochemistry. This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y. The authors wish to express their appreciation to Kurt Salomon for his interest in the investigation.

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