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.

References

1. Branson, H., Banks, H. W., and Dodson, L. B. *Science*, 1947, **106**, 637.
2. Hevesy, G., Levi, H. B., and Rebbe, O. *Biochem. J.*, 1938, **32**, 2147.
3. Holt, M. W., Cowing, R. F., and Warren, S. *Science*, 1949, **110**, 328.
4. Kitson, R. E., and Mellon, M. G. *Ind. eng. Chem.* (Anal. ed.), 1944, **16**, 466.
5. Kugler, O. E. *Am. J. Physiol.*, 1936, **115**, 287.

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.

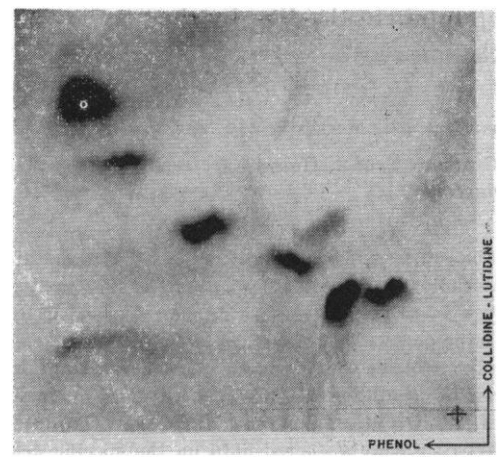

FIG. 1. Paper chromatogram of an apoferritin hydrolysate.

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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spleen apoferritin hydrolysate, equivalent to 0.8 mg of protein, is shown in Fig. 1. The hydrolysis was carried out with 6*N* HCl at 100° C for 18 hr. The following amino acids were present as ninhydrin-reactive substances: tyrosine, phenylalanine, the leucines, valine, glutamic acid, aspartic acid, glycine, serine, arginine, lysine, cystine (as cysteic acid), and methionine. Threonine was observed in trace amounts on chromatograms employing larger quantities of protein. Histidine was not observed in Fig. 1 because of its low sensitivity to the ninhydrin reagent, although its presence was reported by Mazur and Shorr (*5*). The relative quantitative estimations are in agreement with those given by Mazur and Shorr (*5*), except that, in addition to the amino acids listed by these authors, aspartic acid, glycine, serine, and threonine were detected chromatographically.

Paper chromatography of horse spleen ferritin showed the same amino acid composition as that of apoferritin but, in addition, it was observed that some of the iron, liberated by hydrolysis, had migrated in the phenol direction as a definite yellow spot. A chromatogram of ferric iron alone did not show this movement. It was found that the migration of iron on the chromatogram of the ferritin hydrolysate was not characteristic of ferritin, for identical movement was seen in a paper chromatogram run on Amigen (commercial pancreatic casein hydrolysate), to which ferric iron had been added in amounts approximating that contained in ferritin. In all these chromatograms, however, the amino acids moved to their relative positions with the collidine-lutidine solvent, whereas the iron remained on the phenol abscissa. Thus, the iron appears to be conjugated with one or more of the amino acids in the phenol run, but the association is disrupted by the collidine-lutidine solvent.

One-dimensional chromatograms (phenol solvent) of several single amino acids with added iron showed that all the amino acids tested were capable of moving iron from the starting point in varying degrees. Each amino acid could be classified according to the amount of iron it carried (Table 1).

TABLE 1

Amino acid	Amount of iron carried by the amino acid*
Histidine	++++
Alanine	++
Arginine	++
Isoleucine	++
Leucine	++
Lysine	++
Phenylalanine	++
Tyrosine	++
Valine	++
Threonine	+
Serine	+
Aspartic acid	+
Glutamic acid	+
Glycine	+
Control (iron)	No migration

* The system of grading, ++++ to +, is used to designate the amount of iron carried by the amino acid.

Thus, the iron spot, noted on the paper chromatograms of hydrolyzed ferritin and Amigen + iron, is probably due to the fact that such amino acids as histidine, the leucines, arginine, lysine, phenylalanine, and valine are associated with a considerable amount of iron and move to about the same position in phenol.

It is also apparent from Table 1 that histidine conjugates a greater quantity of ferric iron than do other amino acids. Furthermore, the strong affinity of histidine for iron has been reported (*7*). It is possible that a correlation might be found between the histidine content of proteins and their iron-binding capacity.

References

1. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, 1944, **38**, 224.
2. DENT, C. E. *Biochem. J.*, 1947, **41**, 240.
3. *Ibid.*, 1948, **43**, 169.
4. KUHN, R., SORENSEN, N. A., and BIRKOFER, L. *Ber. chem. Ges.*, 1940, **73B**, 823.
5. MAZUR, A., and SHORR, E. *Fed. Proc.*, 1949, **8**, 228.
6. TRIA, E. *Atti accad. It., Rend. classe sci. fisl., mat. nat.*, 1940, **1**, 579.
7. WYMAN, J., JR. In M. L. Anson, and J. T. Edsall (Eds.), *Advances in protein chemistry.* New York: Academic Press, 1948. Vol. IV, p. 410.

Mass Mortality of Fish Associated with the Protozoan *Gonyaulax* in the Gulf of Mexico[1]

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This is the first reported observation of the concurrent appearance of a red tide, luminescent water, and immense numbers of the dinoflagellate protozoan, *Gonyaulax,* with mass mortality of fish on the eastern coast of North America. The episode occurred in the summer of 1949 in a salt water lagoon known as Offatt Bayou along Galveston Island (Figs. 1, 2). Three previously unreported associated biochemical phenomena were revealed by water analyses and offer new clues to the still-undiscovered mechanism of destruction of fish.

From time to time since 1902, comparable but more extensive destruction of fish has been concomitant along the west coast with the appearance of dinoflagellates (particularly *Gonyaulax*), luminescence, and the rusty red water commonly called a red tide (*7, 14*). Similar losses of marine life have occurred in Florida (*4*), but another dinoflagellate, *Gymnodinium,* has been incriminated. Historically, *Gonyaulax* has not only been associated with the mass mortality of marine life, but the species *G. catenella* is known to be the cause of the disastrous poisonings in man by mussels along the Pacific coast (*10–14*). Since 1941, when there were 346 cases with 24 deaths, there have been only a few cases, because state laws forbid the gathering of shellfish during the

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