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Biosynthesis of Phosphoprotein in the Frog. (24412)

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The role of yolk proteins in development of amphibian embryo has been investigated by several workers(1,2). These studies have revealed the important role of phosphoproteins as a source of both amino acids and phosphorus for the growing organism. The isotopic and serological experiments of Flickinger and Rounds(3) carried out with the hen indicated that phosphoprotein is synthesized mainly in liver and is transported to the ovary by way of blood stream. It was therefore considered of interest to investigate whether a similar mechanism is operative in the case of the frog. The present investigation, carried out with the aid of radioactive phosphorus, provides evidence in favour of such an assumption, and demonstrates besides, the presence of a metabolically active phosphopeptide, utilization of which might represent an important step in biosynthesis of phosphoprotein.

Materials and methods. Two normal adult female frogs of similar size and age (*Rana esculenta*) were each given intraperitoneal injection of 100 μc of radioactive phosphorus as sterilized orthophosphate in isotonic saline at pH 7.0. This isotope was purchased from Radiochemical Centre, Amersham, U.K. One frog was killed at end of 6 hours, the other at end of 24 hours. Liver and ovary were removed immediately after sacrifice, and were homogenized in the cold with 10% trichloroacetic acid in a Potter-Elvehjem type of homogenizer. The various phosphorus-containing fragments of the tissues were separated according to procedure of Schneider(4) as modified by Friedkin and Lehninger(5). The acid-insoluble fraction, after exhaustive extraction for lipids, was dried and the phosphoprotein phosphorus estimated according to the method of Schmidt and Davidson(6). Phosphorus estimations in the various fractions were carried out according to the method of Fiske and Subbarow(7). For radioactivity measurements, suitable aliquots of the solution were evaporated to dryness in a planchet and were counted at infinite thinness in a thin end-window Geiger tube (window weight 2.1 mg/cm^2) coupled to a Panax counting equipment (Panax Equipment, Mitcham, Surrey, U.K.).

Results. The results of studies on distribution of radioactive phosphorus in the various fractions of liver and ovary of frog are given in Table I. The results indicate that in the phosphoprotein fraction of liver, there is a very high rate of renewal of phosphorus. This observation is similar to the finding of earlier workers(8,9). Specific activity of this fraction is 4-5 times higher than that of phospholipid fraction. The results also show that rates of renewal of phosphorus in the various fractions of ovary are generally very low.

Analysis of acid-soluble organic phosphates of liver. The high activity of the acid-soluble organic phosphates of liver led to a detailed characterization and analysis of this fraction for the possible presence of any active component that might be involved in biosynthesis of phosphoproteins. For characterization of this fraction, the organic phosphates were precipitated as barium salts as described(10). The free phosphate esters, after removal of barium, were subjected to paper strip electrophoresis in barbital-barbiturate buffer (pH

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TABLE I. Distribution of Radioactive Phosphorus in Various Fractions of Liver and Ovary of *Rana esculenta*.

Fraction	Hr after inj.	Ovary: Total activity of fraction (counts/min.)	Ovary: Specific activity*	Liver: Total activity of fraction (counts/min.)	Liver: Specific activity*
Inorganic phosphorus	6	100,000	113.5	800,000	500.0
	24	127,300	130.5	249,300	265.0
Organic phosphorus	6	10,900	4.6	786,000	142.8
	24	10,400	3.5	102,000	35.8
Phospholipid	6	1,970	.73	98,560	32.2
	24	13,120	4.16	11,200	6.5
Phosphoprotein	6	2,670	.33	27,520	133.3
	24	1,520	.31	13,690	41.6

* Expressed as counts/min./μg phosphorus.

8.6, ionic strength 0.01). The electrophoretic run was conducted for 2 hours at 250 volts at room temperature. Spraying the strip with a modified ninhydrin reagent(11) revealed the presence of 3 ninhydrin positive bands. The strip was exposed to an 'Ilford-Ilfex' X-ray film for 48 hours for detection of radioactive bands.

For further characterization of the active band, the electrophoresis was run in 4 strips, and with the aid of a guide strip, radioactive bands were cut out, eluted with water and hydrolysed with 2 N hydrochloric acid at 110°C for 24 hours in sealed tubes. After removal of acid over potassium hydroxide *in vacuo*, the constituent amino acids in the hydrolysate were analysed by ascending paper chromatography using n-butanol:acetic acid: water (4:1:5) as solvent. Authentic samples of pure amino acids were run alongside as reference spots. The peptide was found to contain the amino acids, leucines, valine, proline, alanine, threonine, serine, glutamic acid and phosphoserine. It may be mentioned that these amino acids also form the constituents of casein phosphopeptone and of the naturally occurring phosphopeptides of the lactating mammary gland(10).

Discussion. Unlike the mammary gland of lactating animals, where both a high rate of renewal of phosphorus of phosphoprotein fraction and synthesis of phosphoprotein are taking place(11), the ovary of the frog, though a store-house of phosphoproteins, renews phosphorus in these proteins at a very sluggish rate. The high rate of renewal of phosphorus in liver phosphoprotein fraction is explained in the observation of Flickinger and Rounds(3), who established liver as the major site of phosphoprotein synthesis in the case of laying hen. Decrease in specific activity of phosphoprotein fraction of liver in 24 hours can thus be due to a rapid synthesis of phosphoprotein in liver, and its subsequent transport to the ovary by way of blood stream, just as in the case of laying hen. But the absence of any significant rise in specific activity of ovary phosphoprotein fraction at the end of 24 hours is rather surprising, and it is quite likely that transport of phosphoprotein from liver to ovary is a slow process in the frog. Incidentally, the results presented in Table I are indicative of a transport of phospholipids also from liver to ovary.

In keeping with the high rate of turnover of phosphorus in the phosphoprotein fraction of liver is the high activity of acid-soluble organic phosphates (Table I). A major constituent of this fraction was identified as a phosphopeptide, which on hydrolysis, yielded the same amino acids as casein phosphopeptone. Failure to detect such phosphorylated peptides in acid-soluble organic phosphates of mammalian liver, and demonstration of their presence in lactating mammary glands(10), strongly suggest that the occurrence of such peptides may be confined to organs actively synthesizing phosphoproteins. Further, the close similarity in the amino acid pattern of the phosphopeptide of frog liver and mammary glands of rat appears to corroborate Perlmann's view(12) that certain types of amino acid sequences around phosphoserine

residue occur in all phosphorus-containing proteins.

Summary. Intraperitoneal administration of radioactive phosphorus to *Rana esculenta* resulted in an appreciable incorporation of the isotope in the phosphoprotein fraction of liver and very much less in the corresponding fraction of ovary. The presence, in liver, of a metabolically active phosphopeptide, was demonstrated. These findings were discussed in relation to the site of synthesis of phosphoproteins in the amphibian.

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Metabolism of I^{131}-Labeled Tobacco Mosaic Virus in Spleen and Liver.* (24413)

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If antigen persistence is essential for long-term immunity, we might expect a low rate of disappearance of antigen from a tissue with high antibody-producing capacity. In experiments reported herein, rate of disappearance of I^{131}-labeled tobacco mosaic virus (TMV) from spleen, an important site of antibody production, was compared with that in liver, which produces little or no antibody(1,2). Persistence of TMV during cell division was also investigated. Metabolism of a viral antigen was studied because of long-lasting immunity reported for certain virus diseases(3). TMV was chosen because, being a plant virus, it is unlikely to multiply in an animal host and complicate study of its localization and fate.

Methods. TMV in the mouse. Labeling procedure was a modification of the technic of Fine and Seligman(4). To 1 ml of carrier-free NaI^{131} (5 mc) was added 0.5 ml of a 1:100 dilution of a stock solution containing 0.05 *M* KI and 0.01 *M* KIO_3; total iodine equivalent added, 38 μg. Free iodine was released by further addition of 1 drop of 3 *M* HCl and extracted with 2 ml of CCl_4. The extract was then slowly added, with stirring, to a solution made by combining 28 mg of TMV in 5.5 ml of cacodylic acid buffer (~pH 6.9) with 15 ml of phosphate buffer (pH 7.5; ionic strength 0.2). After 4 minutes, the aqueous layer was separated and dialyzed for 2 days against four 4000-ml volumes of 0.15 *M* NaCl. After dialysis and subsequent extraction with trichloroacetic acid and organic solvent(5), the dried TMV precipitate contained 0.013% iodine. Three-month-old male (C57BL x 101)F_1 mice were each given 0.6 mg of labeled TMV (5.83×10^5 cpm) via tail vein. Reutilization of released I^{131} was re-

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