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.

1. Barth, L. B., *The energetics of development,* Columbia Univ. Press, 1954,

2. Flickinger, R. A., *J. Exp. Zool.,* 1956, v131, 307.

3. Flickinger, R. A., Rounds, D. E., *Biochim. Biophys. Acta,* 1956, v22, 38.

4. Schneider, W. C., *J. Biol. Chem.,* 1945, v161, 293.

5. Friedkin, M., Lehninger, A. L., *ibid.,* 1949, v177, 775.

6. Schmidt, G., Davidson, H. M., *Biochim. Biophys. Acta,* 1956, v19, 116.

7. Fiske, C. H., Subbarow, Y., *J. Biol. Chem.,* 1925, v66, 375.

8. Davidson, J. N., Frazer, S. C., Hutchison, W. C., *Biochem. J.,* 1951, v49, 311.

9. Agren, G., de Verdier, C-H., Glomset, J., *Acta. Chem. Scand.,* 1954, v8, 1570.

10. Sampath Kumar, K. S. V., Sundararajan, T. A., Sarma, P. S., *Biochim. Biophys. Acta,* 1957, v24, 218.

11. Sampath Kumar, K. S. V., Ph.D. Thesis, Madras Univ., Jan. 1958.

12. Perlmann, G. E., *Advances in Protein Chemistry,* 1955, v10, 1, Academic Press, N. Y.

Received September 12, 1958. P.S.E.B.M., 1958, v99.

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

WALLACE FRIEDBERG† (Introduced by Norman G. Anderson)
Biology Division, Oak Ridge National Lab.,‡ Oak Ridge, Tenn.

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-

* I am indebted to Dr. Irving Rappaport, Dept. of Botany, University of California, Los Angeles, for a gift of TMV. Helpful discussions with Drs. N. G., Anderson, M. A. Kastenbaum, and A. W. Kimball and assistance of Mr. R. E. Canning in some procedures are also appreciated.

† Public Health Service Research Fellow, Nat. Cancer Inst.

‡ Operated by Union Carbide Nuclear Co. for U. S. Atomic Energy Com.

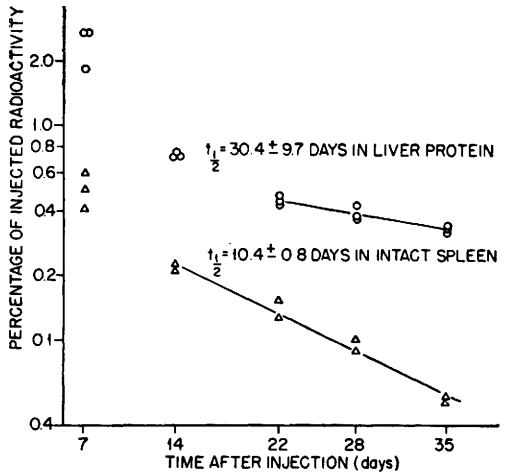

FIG. 1. Radioactivity remaining in liver protein and intact spleen after intravenous inj. of I^{131}-labeled TMV. Upper curve: each point represents protein of one liver; 3 points at each interval. Lower curve: each of the 3 points for the first interval represents one spleen; each of the other points represents avg of 3 intact spleens.

duced by use of 0.003 *M* KI solution as drinking water throughout the experiment. Each mouse also received a 0.5-ml intraperitoneal injection of a 0.03 *M* KI solution 2-3 hrs before the labeled TMV. At appropriate times, mice were killed and spleens and livers removed. Spleens were not further treated. Protein was separated from individual livers essentially as described by Walter *et al.*(5). Radioactivity was measured in well-type scintillation counter and corrections made for physical decay of isotope and background. Counting error (coefficient of variation) was $<4\%$.

Results of mouse experiment. Fig. 1 summarizes studies on radioactivity measurements in mouse spleen and liver. In both tissues, rate of disappearance (slope) of the labeled TMV initially decreased with time and then became constant 2 or 3 wks after injection. At this time, the half life of TMV was about 30 and 10 days in liver and spleen, respectively.

TMV in the rat. To determine whether TMV, localized in a cell, is retained during cell division, its persistence was compared in normal liver of mature rat and regenerating liver of partially hepatectomized rat. Mitotic activity is low in normal liver and high in regenerating liver(6). Iodination was by the procedure already described except that 12 mc of NaI^{131} was used and the TMV was suspended in pH 7.5 phosphate buffer of ionic strength 0.2. Ten Sprague-Dawley rats (280-297 g) were anesthetized and injected with 0.4 mg of I^{131}-labeled TMV (3.3×10^6 cpm) via the saphenous vein. Iodine was given in drinking water as in the mouse experiment, but the rats were started on the iodide water 4 days before TMV was injected. Nine days after virus injection, the rats (avg 311 g) were equally divided into 2 groups. In one group the median and left lobes of liver were removed(7). Seven days later, all rats were bled and killed; the blood was combined with 0.5 ml of 0.1 *M* versene. Radioactivity was measured as described for mice. Counting error in this series was $< 4\%$ except in blood, in which the error was 35%, and the butanol extract of liver homogenate, where the count was insignificant. Radioactivity of blood was determined on 1-ml samples and corrected for the small volume of versene present. Measurements were made on untreated spleen and thyroid, and also on samples of hair (210-360 mg) before and after extraction with 10-ml volumes each of acetone, ethanol, and acetone. The right lobe of liver was homogenized in a volume of 0.14 *M* NaCl equal to its weight, and protein was separated in the same manner as mouse liver protein.

TABLE I. Pooled Liver Lobes—Right Lateral and Small Caudate—16 Days after Injection of I^{131}-TMV in Rats.

		Control lobes (5 rats)	Regenerating lobes (5 rats) (7 days after partial hepatectomy)	% change
Total wt (mg)	Fresh tissue	4,150 ± 309	7,600 ± 528	+83
	Protein	990 ± 61	1,500 ± 65	+52
Total radioactivity (cpm)	Based on tissue	29,000 ± 1100	27,000 ± 4100	− 6.9
	Based on protein	26,000 ± 1400	21,000 ± 3800	−19

TABLE II. Radioactivity in Tissues and Fractions 16 Days after Injection of I^{131}-TMV in Rats.

Tissue or fraction	Control rats (5)	Partially hepatectomized rats (5)
Blood	33 ± 4 cpm/ml	47 ± 6 cpm/ml
Thyroid	345 ± 31 cpm/thyroid	350 ± 31 cpm/thyroid
Hair: Before extraction	5.4 ± .8 cpm/mg	10 ± 2 cpm/mg
Hair: After extraction with acetone & ethanol	5.0 ± .8 "	9.6 ± 1.7 "
Liver protein from pooled right lateral and small caudate lobes	26 ± 1 "	14 ± 2 "
Butanol extract of pooled median and left lateral liver lobes	.045± .009 cpm extracted /mg fresh tissue	.024± .005 cpm extracted /mg fresh tissue (9 days after inj.)

Liver homogenate samples (340-610 mg) were extracted with 5-ml volumes of *n*-butanol. The butanol extract data are presented as extracted activity/mg of tissue.

Results of rat experiment. Increase in liver weight (Table I) in partially hepatectomized rats is obviously significant in terms of tissue and protein. The apparent virus decrease based on radioactivity is not statistically significant ($p > 0.30$) for either tissue or protein.

Data on several tissues and fractions are presented in Table II. Blood specific activity in relation to specific activity of liver shows that radioactivity of liver cannot be accounted for by its blood content. In the thyroid gland, accumulation of I^{131} released from TMV was not completely blocked. Trapped TMV might account for some activity. Radioactivity was not significantly reduced in hair after extraction with organic solvents. A similar finding was reported by Fleischer and Haurowitz(8). Butanol extracts of liver contained no significant amount of radioactivity, indicating that the I^{131} in this tissue is not in the form of loosely bound thyroxine or diiodotyrosine(9).

Data in Table III show that there is no significant increase of spleen weight in rats with regenerating liver. But the disappearance rate of TMV is slightly more rapid from the spleen of partially hepatectomized rats. The level of statistical significance is such that the data indicating increased rate of disappearance, although very suggestive, are not conclusive. In any event, there is at most only a small difference in radioactivity of the spleen and liver between control and partially hepatectomized adult rats.

Discussion. Rate of disappearance of antigen during the period immediately after injection was, in general, consistent with that obtained by Libby and Madison(10) with P^{32}-labeled TMV. In our study, however, attention was focused principally on antigen disappearance rate relatively late after injection, when it appeared to be constant, rather than during the initial period, when the greater part of the antigen was lost rapidly. This period was selected on the assumption that persistence of antigen is essential for lasting immunity.

During the period of constant disappearance rate I^{131}-labeled TMV was metabolized more rapidly in spleen than in liver. Persistence of TMV is therefore not in direct relation to the tissue's antibody-producing capacity. However, antigen may be metabolized in antibody-producing cells of spleen more slowly than in tissue as a whole.

In the regenerating liver, total radioactivity was not significantly influenced by increased mitotic rate. This can be explained by TMV

TABLE III. Spleen 16 Days after Injection of I^{131}-TMV in Rats.

	Control rats (5)	Partially hepatectomized rats (5)	% change	Probability
Fresh tissue, wt	830 ± 48 mg	910 ± 55 mg	+ 9.6	.26
Radioactivity	32,073 ±4053 cpm/spleen	22,161 ±3746 cpm/spleen	−31	.11
Specific radioactivity	39 ± 3 cpm/mg fresh tissue	25 ± 6 cpm/mg fresh tissue	−36	.11

transfer from parent to daughter cells. Some loss of virus during mitosis was not ruled out. TMV-containing cells may not have divided, but this does not seem likely. The role of mitosis in TMV metabolism in other tissues is only suggested by these data. If iodine is transferred from virus to spleen protein, the true disappearance rate of the virus in spleen is even greater than measured.

The possibility of deiodination of intact virus has not been dismissed. *In vitro* experiments of Tong *et al.*(11) indicate that liver can deiodinate diiodotyrosine, but spleen has little or no such ability. The same may be true for deiodination of the virus.

Summary. Rate of disappearance of injected I^{131}-labeled TMV was more rapid from spleen, an important site of antibody production, than from liver, which produces little or no antibody. It was concluded, therefore, that persistence of the virus is not in direct relation to the tissue's antibody-producing capacity. The need for studies at cellular level was indicated. In regenerating liver, total radioactivity was not significantly influenced by increased mitotic rate. This was interpreted as indicating that virus localized in liver may be retained during cell division.

1. Hill, A. G. S., Deane, H. W., Coons, A. H., *J. Exp. Med.*, 1950, v92, 35.

2. Coons, A. H., Leduc, E. H., Connolly, J. M., *ibid.*, 1955, v102, 49.

3. Rivers, T. M., in *Viral and Rickettsial Infections of Man, T. M. Rivers,* ed., Lippincott, Philadelphia, 1948 (see p10).

4. Fine, J., Seligman, A. M., *J. Clin. Invest.*, 1944, v23, 720.

5. Walter, H., Haurowitz, F., Fleischer, S., Lietze, A., Cheng, H. F., Turner, J. E., Friedberg, W., *J. Biol. Chem.*, 1957, v224, 107 (see p110).

6. Brues, A. M., Marble, B. B., *J. Exp. Med.*, 1937, v65, 15.

7. Higgins, G. M., Anderson, R. M., *Arch. Path.*, 1931, v12, 186.

8. Fleischer, S., Haurowitz, F., *Abst. Am. Chem. Soc.*, 1957, p58C.

9. Taurog, A., Chaikoff, I. L., *J. Biol. Chem.*, 1948, v176, 639.

10. Libby, R. L., Madison, C. R., *J. Immunol.*, 1947, v55, 15.

11. Tong, W., Taurog, A., Chaikoff, I. L., *J. Biol. Chem.*, 1954, v207, 59.

Received September 19, 1958. P.S.E.B.M., 1958, v99.

Synthesis of Glucose-Cyclo-Acetoacetate and Biosynthesis of Ascorbic Acid in Germinating Mung Beans (*Phaseolus radiatus*).* (24414)

M. C. NATH AND M. L. BELKHODE
Dept. of Biochemistry, Nagpur University, Nagpur, India

After Nath *et al.*(1) reported that acetoacetate accelerates biosynthesis of ascorbic acid in rats, it was shown that glucose condensed with acetoacetate, can give rise to formation of ascorbic acid in germinating legumes (*Phaseolus radiatus*)(2) and in rats (3). An alternative pathway of conversion of glucose to ascorbic acid has been suggested by Horowitz and King(4) and Isherwood *et al.*(5) in chloretinised rats and germinating seeds respectively. It has been suggested that formation of d-glucuronolactone is an intermediary stage in such transformation. Chel-

* The authors wish to record their grateful thanks to the Council of Scientific & Industrial Research for a Research Grant.

delin and King(6) have, however, put forward arguments which can detract from the overall importance of our scheme. Thangamani and Sarma(7) recently have shown that the yield of radioactive l-ascorbic acid in germinating mung beans from labelled glucose-cyclo-acetoacetate is more than 5 times higher than that from labelled glucose. It has, therefore, been desirable to see if there is any indication of enzymatic synthesis of glucose-cyclo-acetoacetate during the process of germination. Nath and Sahu(8) have shown that glucose occurs in combination with acetoacetate in urine of normal rats thus indicating its formation in the living system. We reported that the condensation product of glu-