compound can be accepted as a vitamin, evidence for its existence in natural foods must be found. Before the growth-promoting effect of L-lyxoflavin can be considered of practical importance, studies must be made with this compound using diets of a more varied nature including a wider range of natural feed ingredients. Studies of this nature are at present in progress.

Summary. In trials with turkey poults highly significant growth responses were obtained with male poults fed a corn-soybean diet supplemented with L-lyxoflavin. Chicks fed purified diets failed to show a growth response when the diet was supplemented with L-lyxoflavin.

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1. Pallares, E. S., and Garza, H. M., *Arch. Biochem.*, 1949, v22, 63.

2. Emerson, Gladys A., and Folkers, K., *J. Am. Chem. Soc.*, 1951, v73, 5383.

3. Wahlstrom, R. C., and Johnson, B. C., *J. Animal Sci.*, 1951, v10, 1065.

4. ———, PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 636.

5. Bruins, H. W., Sunde, M. L., Cravens, W. W., and Snell, E. E., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 535.

6. Cooperman, J. M., Marusich, W. L., Scheiner, J., Drekter, L., DeRitter, E., and Rubin, S. H., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 57.

7. Shorb, Mary S., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 611.

8. Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 469.

9. Sherwood, D. H., and Briggs, G. M., *Poultry Sci.*, 1951, v30, 902.

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Distribution of P^{32} Following the Injection of TEPA* in Rats.† (20370)

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The ethylenimines have been shown to be effective in producing regressions of certain transplantable tumors in rats(1-3). These compounds will inhibit growth and cause cellular destruction of the tumor tissue at concentrations which will not produce any demonstrable changes in normal tissue. At higher concentrations, however, they will inhibit the growth of the testes and white blood cells(2,3). Still higher concentrations will cause general tissue damage. Studies have been started, therefore, to determine the specific effects of these compounds upon the various tissues together with their distribution throughout the body. A preliminary report is presented here of the distribution in rats of P^{32} from N, N′, N″-triethylenephosphoramide (TEPA*).

Materials and methods. Fifty-eight Sprague-Dawley male rats (175-200 g) were divided into 3 groups, 2 of which were implanted subcutaneously with the Flexner-Jobling carcinoma, the third group serving as a control. Ten days after transplantation, each of 24 rats in Group A bearing the tumors received one dose subcutaneously of TEPA*, 5.0 mg/rat, (900 μg P/rat), (1.55×10^4 CPM/μg P). In Group B the 16 control rats were treated with TEPA* at the same dosage level as the rats in Group A. The 18 rats in Group C bearing the tumors were injected subcutaneously with Na_2HPO_4*, 4.3 mg/rat, (400 μg P/rat), (7.16×10^3 CPM/μg P). At 1, 2, 15, and 30 hours following injection of the tagged compound, several animals from each group were autopsied. Samples of liver,

* Radioactive.

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TABLE I. Distribution of P^{32} after Injection of TEPA* or Na_2HPO_4* into Rats. Each average represents data from 6 animals in the tumor groups and four in the control groups.

	Interval after injection											
	1 hr			2 hr			15 hr			30 hr		
	μg P^{32} per g of wet tissue											
Group	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min
TEPA*-Control												
Liver	3.3	4.6	2.4	3.0	3.5	2.0	2.9	3.0	2.8	2.5	3.0	1.7
Testis	3.6	4.6	1.9	4.0	4.7	3.7	1.4	1.7	1.2	1.0	1.2	0.7
Kidney	4.8	6.1	3.3	4.9	6.4	3.5	1.3	1.4	1.2	1.2	1.4	1.1
Spleen	2.4	3.3	1.8	2.7	2.9	2.5	4.8	6.1	4.1	7.0	8.3	5.8
Bone	1.9	2.3	1.0	1.9	2.3	1.8	4.5	4.8	4.0	4.8	6.2	3.5
TEPA*-Tumor												
Liver	3.6	4.8	2.5	2.9	4.3	2.0	2.6	3.3	2.0	2.6	3.1	2.0
Testis	2.5	4.9	0.5	3.1	5.1	0.6	1.1	1.5	0.6	0.9	1.1	0.7
Kidney	5.5	8.1	4.1	6.3	14.4	1.4	2.3	2.8	1.6	1.6	2.6	1.0
Spleen	2.5	4.3	0.4	2.8	3.9	0.8	2.8	3.4	2.1	2.9	4.7	1.9
Bone	2.5	5.7	1.3	2.2	3.3	1.0	4.5	5.4	3.9	5.3	6.0	4.7
Tumor	2.7	4.0	0.5	2.4	4.1	1.2	2.1	2.4	1.8	3.3	4.8	2.1
Na_2HPO_4*-Tumor												
Liver	8.3	10.0	7.4	6.7	10.0	3.8	3.0	4.2	2.4			
Testis	.4	.5	.3	.4	.7	.1	.1	.3	0			
Kidney	10.0	11.1	6.0	4.4	5.5	3.7	1.8	2.0	1.6			
Spleen	4.3	5.6	3.7	2.9	3.6	2.3	2.3	3.0	1.8			
Bone	9.4	11.0	5.1	13.9	18.7	9.7	13.2	15.5	12.1			
Tumor	4.9	5.9	4.6	3.0	4.2	3.3	2.5	3.0	2.0			

* Radioactive.

kidney, testes, spleen, bone, and tumor were taken for assay. Weighed samples of each tissue were digested in a mixture of HNO_3 and H_2SO_4, 1:1, and 100 λ aliquots of the digest were counted with an end window (1.4 mg/cm^2) GM counter. Urine and fecal samples were collected from 6 rats of Group A and 4 rats of Group B for 24 hours following injection of the tracer. The samples were pooled within each group and prepared for counting as described above. Blood samples were obtained at the 2-hour interval from 6 rats in Group A and 4 rats in Group B and pooled within each group. Both plasma and serum were prepared and fractioned as follows. The globulins were precipitated by ½ saturation with $(NH_4)_2SO_4$. The remaining proteins were precipitated, first with sulfosalicylic acid and then phosphotungstic acid. P^{32} activities were determined on these 3 resultant filtrates as well as on the original serum and plasma. Samples of the liver, testes, and tumor obtained from Groups A and B at the 2-hour interval were fractioned for cellular components according to Schneider and Hogeboom(4). To do this, tissue was homogenized with ice cold (0.25 M) sucrose solution. The homogenate was centrifuged for 10 minutes at 700 x gravity, a procedure that separates nuclei from cellular components in liver tissue. Similarly, the mitochondria of liver cells were separated by centrifugation at 5000 x gravity and the microsomes at 25,000 x gravity. Since cellular particles have not been studied in detail in the testis and tumor tissues, the particles separated by this procedure will be characterized in terms of gravity.

Results. Distribution in tissues. The distribution of P^{32} following injection of tagged TEPA* or Na_2HPO_4* in various tissues of the rat is recorded in Table I. These data illustrate the rather uniform distribution of P^{32} activity from TEPA* throughout the body including the tumor. However, the P^{32} following injection of TEPA* concentrated more rapidly in the testes and less rapidly in bone than following injection of inorganic phosphate. There was a marked increase in activity in the spleen over a period of 29 hours (2.4 to 7.0 μg P^{32}/g spleen) in normals receiving TEPA*. The average size of the spleen was 300 mg/100 g body weight making an average of 2100 μg of P^{32} in the entire spleen

TABLE II. Distribution of P^{32} following Injection of TEPA* in Cellular Fractions in % of Total Activity. Particle size recorded in terms of gravity used for centrifugation.

Animal	Tissue	700 × gravity	5000 × gravity	25000 × gravity	Soluble
Normal	Liver	20.0	4.8	6.7	68.5
Tumor-bearing	"	18.3	5.8	5.4	70.1
Normal	Testis	22.2	3.2	4.2	70.5
Tumor-bearing	"	27.4	2.5	2.1	68.0
"	Tumor	15.2	3.8	4.3	76.5

* Radioactive.

30 hours following injection of TEPA*. The μg of P^{32}/g of spleen on the other hand, remained essentially constant in tumor-bearing animals. The size of the spleen, however, was increased in these animals to 900 mg/100 g body weight making the total activity at the end of 30 hours 2610 μg P^{32}, almost identical with the normal spleens. Concentration of P^{32} from TEPA* in the spleen may be associated with specific effects on the reticuloendothelial system.

Distribution in the cells. One type of measurement of the distribution of P^{32} from TEPA* in liver, testes, and tumor cells is illustrated in Table II. These data indicate a combination of P^{32} with the nuclei and/or other relatively heavy cell components as separated by centrifugation at 700 x gravity. Very little activity was found in lighter particles separated by 5000 or by 25,000 x gravity. Approximately 70% of the activity remained in the soluble fraction not thrown out by centrifugation. This soluble P^{32} remained in the filtrate after precipitating proteins with phosphotungstic acid.

Distribution in plasma. Two hours after injection of TEPA*, tagged with P^{32}, blood samples were taken from control and tumor-bearing rats. Approximately 60% of the P^{32} activity of either serum or plasma was precipitated from solution with the globulin fraction. The remaining 40% was in solution even after precipitating the proteins and polypeptides with sulfosalicylic acid and phosphotungstic acid. These results together with those presented previously suggest that TEPA* can combine with certain proteins, possibly thereby inhibiting some metabolic pathways.

Excretion. The activity in the excreta collected over a period of 24 hours following the injection of TEPA* was identical in normal and tumor-bearing animals. In both groups, 62% of P^{32} was excreted in the urine and 5% in the feces. It is possible that the small amount in the feces came from contamination with urine since an exact separation of these two products of excretion is difficult. These data demonstrate, however, that a large portion of the P^{32} has been excreted from the body 30 hours following a single injection of TEPA*.

Summary. The distribution of P^{32} following the injection of tagged TEPA* resulted in a fairly uniform distribution of P^{32} activity throughout the tissues of the body, but there was a greater concentration in the testes and a smaller concentration in bone as compared with injection of inorganic phosphate. There was a gradual accumulation of P^{32} in the spleen over a period of 30 hours following injection of TEPA*, a result not observed with inorganic phosphate. Approximately 60% of the P^{32} activity in serum or plasma was associated with the globulin fraction, the remainder being in solution after precipitation of all proteins. About 20% of P^{32} was associated with nuclei and/or relatively heavy cellular components from liver, testes, and tumor tissue, most of the remaining activity being found in the protein-free filtrate. Some 65% of the activity was excreted over a period of 30 hours.

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1. Crossley, M. L., Allison, J. B., Wainio, W. W., and Muenzen, J. B., *J. Nat. Cancer Inst.*, 1951, v12, 305.

2. Crossley, M. L., Allison, J. B., and Muenzen, J. B., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 452.

3. Crossley, M. L., Allison, J. B., Parker, R. P., Kuh, E., and Seeger, D. R., in press.

4. Schneider, W. C., and Hogeboom, G. H., *Cancer Research*, 1951, v11, 1.

Received May 26, 1953. P.S.E.B.M., 1953, v83.