differences in the relative numbers of erythroblastic cells in the spleens of the 3 experimental groups, *i.e.*, a relatively large number that were actively synthesizing hemoglobin in the bled rats, a smaller proportion in the normal animals, and practically none in the hypertransfused rats. The inability of the sheep plasma ESF to induce either ALA dehydrase synthesis in spleen homogenates and ribosome fractions, or enhance ALA dehydrase activity of spleen, marrow and liver soluble fractions implies that this relation was not due to a direct action of ESF upon this enzyme system. The basic action of ESF is best interpreted as due to its ability to increase the *numbers* of hemoglobin synthesizing cells in the spleen and bone marrow.

Summary. The delta-aminolevulinic acid dehydrase activity of rat and mouse spleen, bone marrow and liver fractions and homogenates was determined. Spleen ALA dehydrase specific activity of normal, polycythemic, and anemic rats was found to decrease exponentially with the increase in hematocrit of the animal. However, sheep plasma erythropoietin, incubated with the tissue fractions and homogenates, did not directly produce an increase in enzyme activity over that of the controls.

1. Alpen, E. L., Cranmore, D., in *The Kinetics of Cellular Proliferation,* Frederick Stohlman, Jr., Ed., Grune & Stratton, Inc., New York & London, 1959, p290.

2. Erslev, A. J., *Ann. N. Y. Acad. Sci.,* 1959, v77, 627.

3. Gordon, A. S., *Physiol. Rev.,* 1959, v39, 1.

4. London, I. M., *Bull. N. Y. Acad. Med.,* 1960, v36, 79.

5. Gibson, K. D., Neuberger, A., Scott, J. J., *Biochem. J.,* 1955, v61, 618.

6. Fischer, S., in *Erythropoiesis,* L. O. Jacobson & M. Doyle Eds., Grune & Stratton, Inc., New York and London, 1962, p204.

7. Cookson, G. H., Rimington, D., *Biochem. J.,* 1954, v57, 476.

8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.,* 1951, v193, 265.

Received June 29, 1964. P.S.E.B.M., 1964, v117.

Water Absorption from the Intestine Via Portal and Lymphatic Pathways in Rats.* (29569)

AHMET NOYAN† (Introduced by H. A. Kornberg)
Biology Laboratory, Hanford Laboratories, General Electric Co., Richland, Wash.

It is generally accepted that water is absorbed from the intestine via the portal venous system. However, the study of Kim and Bollman(1) suggested the possibility that the intestinal lymphatic system might be another significant pathway of water absorption. To test this possibility Benson *et al*(2) conducted experiments on rats and showed that at least 99% of the water absorbed from small intestine was carried *via* portal venous system.

Lee(3) recently provided contradicting experimental evidence, *in vitro,* showing that the portal system may not be the major route for water absorption. Segments (15-25 cm) of the rat's small intestine were prepared by cannulating the lymphatic and blood vessels. His results showed that 85% of the absorbed water entered the lymphatics and that only 11% was absorbed by the portal vein.

This paper reports experiments with tritium oxide designed to measure the quantities of water absorbed into both the lymphatic and portal channels in the intact rat.

Methods. Twenty-seven adult Sprague-Dawley female rats averaging 200 g body weight were anesthetized with sodium pentobarbital (32.5 mg/kg). Cannulation of the mesenteric lymph duct was done according

* Work performed under Contract No. AT(45-1)-1350 between Atomic Energy Commission and General Electric Co.

† IAEA Fellow on leave from Dept. of Physiology, Univ. of Ankara, Turkey.

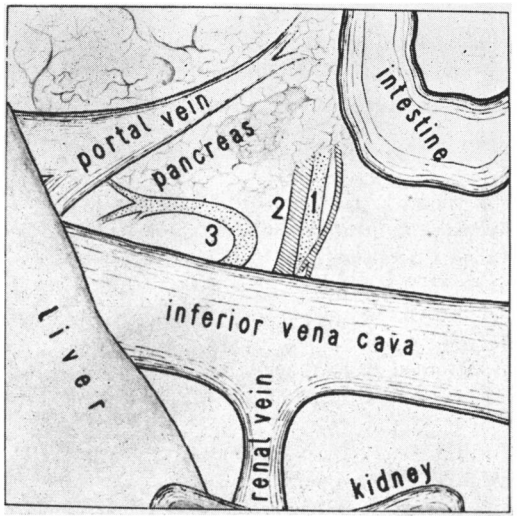

FIG. 1. Anatomical relationship of lymph vessels and organs. (1) Mesenteric artery, (2) mesenteric lymph duct, (3) hepatic artery.

to a procedure similar to that of Bollman, Cain and Grindlay(4) by inserting a cannula into the duct with the help of a dissection microscope (Fig. 1). The cannula consisted of 3 sections of polyethylene tubing with increasing diameters, P.E. 50‡ (0.058 I.D.), P.E. 90, and P.E. 190. Accessory lymphatics were ligated after it was determined that the flow from them was negligible.

Krebs' bicarbonate buffer(5) containing 6.8 μc of tritium/ml was perfused through the lumen of the small intestine at 37°C. A continuous flow was provided by a Sigmamotor pump at a rate of 1.0 ml/min and the perfusion time was for 60 or 90 minutes. The afferent cannula entered the proximal duodenum and the efferent drained the distal ileum. Collection of the perfused solution was made after a single cycle through the intestine and will be designated "recovered Krebs solution." Blood was withdrawn by cardiac puncture to determine when an equilibration of radioactivity was reached between blood and tissue water.

The animals were killed at the conclusion of the perfusion period and analyzed for their tritium content by immersing tissue sections in benzene and distilling the benzene-water mixture at 65°C. All distillations were carried to completion to avoid errors due to isotopic fractionation(6). In the 4 initial experiments, 15 g portions of tissue were dehydrated by this procedure, but for succeeding ones the entire carcass was distilled. Blood and lymph were also dehydrated by this procedure. Tritium activity was determined in a liquid scintillation beta counter equipped with anticoincidence circuitry and cooled photomultiplier tubes. The sensitivity was plus or minus 7%.

‡ Clay-Adams Inc., New York.

The nomenclature used in this report is that of Code(7). "Insorption" refers to the movement of material from the lumen of the intestine into the blood while "exorption" denotes movement in the opposite direction. Absorption describes that portion retained in the body.

Results. Concentration of tritium oxide in tissue water from the whole-body and from a 15 g tissue sample were generally consistent with results obtained by Thompson(8), who found that tritium oxide concentration in the body water did not vary among different organs and tissues of the same animal. The body water content of rats in our experiments ranged from 57 to 64% of body weight (average 61%).

Table I shows that tritium concentration in blood and tissue water reached practical equilibration in a period of less than an hour.

The amount of water insorbed was determined by subtracting the radioactivity of the recovered buffer from the amount perfused into the intestine. The average activity density perfused was 6.8 μc of tritium/ml. If there had been no net transfer of tritium from the perfusate the 46 ml of Krebs' solution recovered should have contained about 310 μc of tritium; however, only 140 μc was collected. This indicated that the net flow of water was in the direction of the blood and that dilution of the perfusate resulted by exorption of non-radioactive water from the blood stream.

Water insorption, absorption and exorption data, under these experimental conditions, were calculated from tritium concentrations in perfused and recovered solutions and are shown in Table II. Although these

TABLE I. Tritium Concentration in Blood, Tissue, and Lymph Following Perfusion of the Intestine with Tritiated Buffer.

No. of rats	Perfusion time (min)	Blood H^3 activity (μc/ml)	Tissue H^3 activity (μc/ml)	Lymph H^3 activity (μc/ml)	Lymph Vol. collected (ml)
7	60	2.1 ± .4*	1.7 ± .3	1.6 ± .2	4.7 ± .7
7	90	1.6 ± .2	1.8 ± .1	1.5 ± .2	5.2 ± .4

* Mean values and standard deviations.

calculations should have been derived by extrapolating the exponential equilibration curve back to zero time, when there was no tritium movement, it was not possible under our experimental conditions. Nevertheless, the data do show a significant dilution of recovered solutions by exorbed water.

About 96% (250 μc) of the tritium activity leaving the perfusate was recovered by distillation of the carcass, lymph and blood sample. Of this amount, 240 μc was in the body water. Therefore, 96% of the tritium activity absorbed from the small intestine entered the blood stream and deposited in the tissue. The radioactivity of the lymph collected during the perfusion periods averaged 7.6 μc (Table I), which amounts to about 3% of the total recovered. The average volume of lymph collected was about 5 ml, which accounts for 13% of the quantity insorbed, 38 ml, during the one hour perfusion period. However, all the lymph collected probably did not come from the perfusate since the lymph was milky white during the early periods of collection. It soon cleared, indicating that the source of lymph was from the fat-free Krebs' solution but the delay does not permit our use of lymph volume as a basis for calculations of water absorption.

Discussion. We used Krebs' bicarbonate buffer(5) containing 0.5% glucose as a perfusion medium in the present experiments because Smyth and Taylor(9) have shown it to be favorable for studies of water transport. Fisher(10,11) showed water transport by *in vitro* intestine to be relatively independent of hydrostatic and osmotic forces. Shields and Code(12) found that an increase in portal venous pressure had little effect on water transport in dog ileum. These findings suggest that pressure changes on either the mucosal or serosal side of the intestine exert little influence on water absorption. From this we conclude that the *in vitro* findings of Fisher or Lee(3) may be compared to the present *in vivo* experiments. Since Lee cannulated the lymphatic and mesenteric blood vessels in his *in vitro* preparation and determined the amount of water entering them from the small intestine, such a comparison is desirable. Absorption under Lee's *in vitro* conditions was less than in these studies, although the volume of fluid perfused through intestinal loop in his experiments was 20-30 times greater. This is in accord with findings of Parsons(13) who showed that water transport *in vivo* exceeds *in vitro* results. A limited ATP reserve *in vitro* may serve as a further explanation since water transport is an active process(13). This difference in amount of water transported is not the sole discrepancy between *in vivo* and *in vitro* transport. Lee showed that 85% of water absorption occurred via the lymphatics and that only 11% passed into the portal venous channel. Our finding of 3 and 97% absorption into lymphatic and venous routes is a contradiction to those results obtained by *in vitro* methods.

TABLE II. Amount of Water Insorption, Exorption and Absorption.

Insorption, ml	Exorption, ml	Absorption, ml
38 ± 4.1*	26 ± 5.4	12 ± 3.9

* Mean and standard deviations of 14 samples.

Lee suggested the possibility that water may be first absorbed into the lymphatics and then transferred to vascular system while still in the intestine. Benson *et al*(2), however, states that lymph and the blood derive their water from a common supply because the quantity of D_2O in the lymph and the blood

was the same at all times after intragastric or intravenous administration. These results suggest that the mechanism for water absorption *in vivo* is not analogous to *in vitro* conditions. The major route for transport from the small intestine is *via* the portal venous system and only about 3% is transported *via* the lymphatics.

Summary. When tritiated water was introduced into the lumen of the intestine of rats equilibrium with body water occurred in about an hour. Collections of absorbed tritium in the lymph and body water showed that 97% was absorbed into the portal system and only 3% into the mesenteric lymph.

The author wishes to express his gratitude to Dr. M. F. Sullivan for his suggestions and comments in preparation of this manuscript.

1. Kim, K. S., Bollman, J. L., *Am. J. Physiol.*, 1954, 179, 273.
2. Benson, J. A., Jr., Lee, P. R., Scholer, J. F., Kim, K. S., Bollman, J. L., *ibid.*, 1956, v184, 441.
3. Lee, J. S., *ibid.*, 1961, v200, 979.
4. Bollman, J. L., Cain, J. C., Grindlay, J. H., *J. Lab. Clin. Med.*, 1948, v33, 1349.
5. Krebs, H. A., Henseleit, K., *Z. Physiol. Chem. Hoppe-Seyler's*, 1932, 210, 33.
6. Pinson, E. A., *Physiol. Rev.*, 1952, v32, 123.
7. Code, C. F., *Perspectives in Biol. Med.*, 1960, v3, 560.
8. Thompson, R. C., *J. Biol. Chem.*, 1953, v200, 731.
9. Smith, D. H., Taylor, C. B., *J. Physiol.*, 1957, v136, 632.
10. Fisher, R. B., *ibid.*, 1954, v124, 21.
11. ———, *ibid.*, 1955, v130, 655.
12. Shields, R., Code, C. F., *Am. J. Physiol.*, 1961, v200, 775.
13. Parsons, B. J., *J. Physiol.*, 1959, v148, 117.

Received June 30, 1964. P.S.E.B.M., 1964, v117.

Effect of Dietary Selenium on Incidence of Nutritional Encephalomalacia in Chicks.* (29570)

BERNARD CENTURY AND M. K. HORWITT
L. B. Mendel Research Laboratory, Elgin State Hospital, Elgin, Ill., and Department of Biological Chemistry, University of Illinois College of Medicine, Chicago, Ill.

Extremely low levels of dietary selenium have been shown to ameliorate a variety of conditions in animals fed tocopherol-deficient diets. These include prevention of necrotic liver degeneration(1,2), and delay in onset of creatinuria in vitamin E-deficient rats(3). In tocopherol-deficient chicks, less than 0.1 ppm prevented exudative diathesis(4-6), while higher levels reduced the incidence of muscular dystrophy(4,7). On the other hand, selenium has been reported to be ineffective in preventing encephalomalacia(8-10). Nutritional encephalomalacia in chicks is unique in that it has been related specifically to ingestion of essential fatty acid sources(11-16), rather than to intake of polyunsaturated lipids in general. In the present study, an incidence of encephalomalacia of about 50% was obtained by feeding a diet containing 4% corn oil from which tocopherols had been removed. Under these conditions, a low level of selenium added to the diet was effective in delaying the onset of encephalomalacia.

Materials and methods. One-day-old Vantress-Arbor Acres crossbred cockerels were reared in electrically heated brooders and fed the diet described by Bosshardt *et al* (17), with slight modifications(12). Corn oil was stripped of most of the tocopherols by vacuum distillation† followed by aeration at 100°C until a peroxide number of 60 was reached. The diets containing corn oil at either 4% or 8% levels were isocalorically constant with respect to the protein, vitamins and minerals. Various levels of sodium selenite were added to the vitamin mixture when included in the diet. The corn oil and vitamin mixtures were added to stock mixtures

* This investigation was supported by a U. S. Public Health Service grant and by Illinois Mental Health Fund.

† Distillation Products, Inc., Rochester, N. Y.