inbred mice obtained from abroad, we have found one infected colony among a total of 12.

In the laboratory to which this infected colony belongs, no studies involving polyoma virus have been done, and hence infection through experimental work is out of the question. Furthermore, strains A and DD have been kept in one room, and the other 4 strains in another room. Under the circumstances, infection through keeping uninfected mice in the same room with infected mice seems hardly worth considering. The 4 infected strains (A, CBA, C_3H, and Hr^{rh}) in this colony all came from the United States, but the 2 uninfected strains (DM and DD) did not. We assume that the appearance of polyoma virus antibody in Japan is due to some change in conditions after latent infection. Periodic surveys for polyoma virus antibodies in Japan are probably desirable.

Summary. Twelve mouse colonies in Japan were surveyed for polyoma virus infection. Among them, one colony was found to be infected with polyoma virus, half of the tested mice from that colony being antibody-positive. The infected mouse strains all came originally from the United States, and it is thought that they may have acquired latent infection before being brought into Japan.

1. Stewart, S. E., Eddy, B. E., Irwin, M., Lee, S., *Nature,* 1960, v186, 615.

2. Rowe, W. P., Hartley, J. W., Law, L. W., Huebner, R. J., *J. Exp. Med.,* 1959, v109, 449.

3. Rowe, W. P., *Bact. Rev.,* 1961, v25, 18.

Received October 22, 1962. P.S.E.B.M., 1963, v112.

Inulin and Sucrose Distribution in Tissues of Vitamin E-Deficient and Control Rabbits.* (27984)

J. F. DIEHL AND JOE K. BISSETT

Department of Biochemistry, University of Arkansas Medical Center, Little Rock

The extracellular space in a particular organ or tissue is commonly measured by determining the accumulation of substances that are believed not to occur intracellularly. Determination of tissue chloride concentration has frequently been used for this purpose(1), and skeletal muscle chloride has been found to be increased in the nutritional muscular dystrophy which is caused by vitamin E deficiency. Fenn and Goettsch(2) reported a 3-fold, and Morgulis and Osheroff (3) reported a 2-fold increase of chloride concentration in dystrophic rabbit muscle. While the former authors attributed this increase to an increase in interstitial fluid, the latter authors pointed out that chloride is a component of the connective tissue. They associated the higher chloride content with an increase of fibrous tissue which is characteristically observed in dystrophic muscle.

* Supported by research grant from Nat. Inst. Health, U.S.P.H.S.

The complete interpretation of protein turnover studies with carbon-14 labeled amino acids in dystrophic rabbits(4) requires information on the size of intracellular amino acid pools. Such information cannot be obtained without a knowledge of the size of the fluid compartments in dystrophic and normal muscle. The present report is concerned with the determination of volume of distribution of inulin-C^{14} and sucrose-C^{14} in several tissues of vit. E-deficient and control rabbits under *in vivo* conditions. Other authors have used these saccharides to measure extracellular space (*e.g.,* 5).

Methods. White New Zealand rabbits of mixed sex, approximately 4 weeks old and weighing 500-600 g, were placed on a semisynthetic vit. E-deficient diet. The composition of the diet has been described by Young and Dinning(6) and has been modified to contain cellulose as roughage(7). Animals designated as controls received oral supplements of 12 mg DL-alpha tocopherol acetate

per kg body weight 3 times weekly, as a solution containing 40 mg of α-tocopherol/ml of corn oil. To minimize the effect of possible coccidiosis infection on their vit. E reserves(8) all animals received .04% sodium sulfaquinoxaline in the drinking water for 3-day periods alternating with 2-day periods of plain water. When the vit. E-deficient animals showed distinct signs of muscular weakness, 3 to 4 weeks after they were placed on the diet, they and the control animals which had been on the diet for the same length of time, were selected for the experiment. Bilateral kidney ligation was performed under ether anesthesia. Half an hour after completion of the operation carboxyl-C^{14}-labeled inulin (New England Nuclear Corp.; 2.02 mc/g) or sucrose-U-C^{14} (New England Nuclear Corp.; 5.0 mc/mmole) was injected by ear vein at a dose of 2.5 μc/100 g body weight, in a concentration of 25 μc of saccharide per milliliter of saline. The animals were sacrificed 3 hours after the injection by a sharp blow to the neck and decapitation. Blood was collected in a heparinized beaker. Heart, one kidney and 5 g portions of liver and skeletal muscle from the left hamstring group were rapidly excised, freed of extraneous tissue, blotted on filter paper and weighed. Each tissue was homogenized in a Virtis blender with 20 ml of .01 N acetic acid and rinsed into a centrifuge tube with another 20 ml. The homogenate was heated in a boiling water bath for 10 minutes. The supernatant fluid obtained by centrifugation was filtered and the sediment was washed 4 times with 20 ml portions of the acetic acid. A fifth washing extracted no measurable amount of radioactivity from the residue. All filtrates of one tissue were combined and one ml aliquots were placed in planchets, dried under a heat lamp, and counted in a thin-window gas-flow Geiger counter. Preliminary experiments using water for the extraction instead of dilute acetic acid led to essentially the same results but the extracts were more difficult to filter. Plasma was diluted 1:50 with water and its radioactivity determined as described for tissue extracts.

To study possible effects of starvation on the tissue distribution of inulin, food and vitamin supplements were withheld from rabbits which had previously been on the vit. E-supplemented diet for 3 to 4 weeks. Drinking water was supplied *ad libitum* during the fasting period. Kidney ligation was performed 3 days after withdrawing food. Inulin-C^{14} was injected and tissues were extracted as described.

TABLE I. Distribution of Inulin-Carboxyl-C^{14} and Sucrose-U-C^{14} in Tissues of Vitamin E-Deficient, Starved and Control Rabbits.

	Skeletal muscle	Liver	Heart	Small intestine
Inulin space				
Control (4)*	5.5†	20.9	14.7	5.5
	5.0- 6.1	8.2-38.1	12.3-16.5	4.8-6.8
Vitamin E-	9.8	44.8	17.0	6.3
deficient (4)	8.3-11.6	31.2-76.4	15.2-19.6	4.9-7.5
Starved (2)	5.4	22.3	11.4	6.3
	4.9- 5.8	17.7-29.0	10.3-12.4	5.1-7.5
Sucrose space				
Control (3)	7.4	10.3	18.7	7.2
	6.7- 8.6	7.6-14.5	16.2-22.7	4.7-8.7
Vitamin-E-	12.0	13.8	20.0	6.4
deficient (3)	10.8-14.9	11.5-18.2	14.7-22.9	5.2-7.3

* Number of animals.

† Average $\frac{\text{counts/min/g tissue}}{\text{counts/min/ml plasma}} \times 100$, and range.

Results were calculated as the volume of distribution of the saccharide between tissue and plasma and were expressed as per cent of wet tissue weight(9).

Results and discussion. Results are summarized in Table I. In dystrophic muscle, average sucrose space was increased by a factor of 1.6 and average inulin space by a factor of 1.8. The difference between the vit. E-deficient and control groups was highly significant ($P<.005$) after inulin injection and significant ($P<.05$) after sucrose injection. As young rabbits usually lose weight in the acute stage of vit. E deficiency, the question arose whether this difference between control and vit. E-deficient animals might not be due to the status of general inanition, rather than to vit. E deficiency *per se*. The data obtained on rabbits starved for 3 days indicate that inanition alone had no effect on inulin space of muscle.

No statistically significant differences between control and vit. E deficient or starved groups were found in any other tissue,

whether inulin or sucrose were used. Average inulin space in the liver of vit. E-deficient animals was twice as great as in controls but individual variations in both groups were so great that the difference is of questionable significance ($P = 0.1$). In skeletal muscle, heart and small intestines average sucrose space was consistently somewhat greater than average inulin space. This agrees with observations of others and may be considered either as an indication that cell membranes are not completely impermeable to sucrose or that inulin is restricted from some portion of the extracellular space.

In contrast, average inulin space of liver was much higher than average sucrose space. In the vit. E-deficient group inulin space was as high as 76.4% of liver wet weight. If the volume of inulin distribution were a true measure of extracellular space, all of the water in this liver would have been extracellular water. These livers appeared grossly normal and it seems most unlikely that inulin distribution was truly correlated to extracellular space in this instance. Considerable intracellular accumulation of inulin must be suspected. A possible explanation for the high inulin concentration found in livers of vit. E-deficient animals and in livers of some control and starved animals may be contained in a finding by White and Rolf(10). These authors reported that after inulin injection into nephrectomized rats, inulin space continually increased and eventually greatly exceeded water content in liver, skin, and spleen, tissues rich in macrophages, while it remained less than water content in skeletal muscle, which is poor in macrophages. It was postulated that macrophages actively ingest and concentrate inulin. Unfortunately, histological examinations of the livers were not performed in the present study. It may be that macrophage content of those livers which showed a particularly high inulin content was elevated, possibly as the result of unrecognized infections or inflammatory processes. Gross symptoms of liver coccidiosis were absent. Whether the macrophage content of livers is increased in vit. E-deficient rabbits, possibly as a less drastic expression of processes leading to liver necrosis in vit. E-deficient rats(11) appears to deserve further exploration.

The same comments apply to the inulin space of muscle. Necrosis and appearance of phagocytes have been observed in degenerating muscle of vit. E-deficient animals(12). Theoretically, the higher inulin space of dystrophic muscle could be the result of macrophage accumulation. However, in contrast to liver, skeletal muscle of vit. E-deficient animals showed a significant increase in sucrose space as well as in inulin space. It is not clear whether this increased sucrose and inulin space is caused by an actual increase in the extracellular fluid compartment, or whether vit. E deficiency increases muscle cell membrane permeability to these compounds, leading to some intracellular accumulation. Burr and McLennan(13) found inulin spaces of over 90% in muscle of dystrophic mice and concluded that inulin cannot be confined to the extracellular compartment in this condition. Various changes in vit. E-deficient animals have been attributed to loss of membrane integrity(14). Certainly, it may be concluded from our results that, if there was an increase in extracellular space in muscle of the vit. E-deficient rabbits, this increase was not greater than 2-fold. Assuming that intracellular space is the difference between tissue water content† and inulin space or sucrose space, it is easily calculated that intracellular space in skeletal muscle of the dystrophic animals was decreased by less than 10%. It is known that variations in intensity and duration of vit. E deficiency can lead to quite different pathological pictures(12). It may well be that more pronounced effects on fluid compartment relationships of skeletal muscle would be observed in rabbits of different age or on a different vit. E-deficient diet.

Summary. Inulin and sucrose space were determined under *in vivo* conditions in 4 tissues of vit. E-deficient and vit. E-supplemented rabbits, and inulin space was determined in the same tissues of starved rabbits.

† In agreement with earlier reports(2,3) we have found no significant difference between the skeletal muscle water content of dystrophic and control rabbits.

In vit. E-deficient, but not in starved animals, a significant increase in inulin and sucrose space of skeletal muscle was found. Whether this increase reflected an actual increase of the extracellular fluid compartment or was caused by increased permeability to the saccharides, could not be established. It was concluded that if there was an actual increase of extracellular space in the dystrophic muscle, this increase was not greater than by a factor of 2. Inulin space in livers of all 3 experimental groups was very variable and in most cases much higher than average sucrose space.

The authors wish to acknowledge the capable technical assistance of Mrs. T. E. Ashcraft.

1. Manery, J. F., *Physiol. Rev.*, 1954, v34, 334.
2. Fenn, W. O., Goettsch, M., *J. Biol. Chem.*, 1937, v120, 41.
3. Morgulis, S., Osheroff, W., *ibid.*, 1938, v124, 767.
4. Diehl, J. F., Sanders, L. L., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 8.
5. Eichhorn, J., Halkerston, I. D. K., Feinstein, M., Hechter, O., *ibid.*, 1960, v103, 515.
6. Young, J. M., Dinning, J. S., *J. Biol. Chem.*, 1951, v193, 743.
7. Diehl, J. F., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 657.
8. Diehl, J. F., Kistler, B. G., *J. Nutrition*, 1961, v74, 495.
9. Eichhorn, J., Hechter, O., *J. Gen. Physiol.*, 1961, v45, 15.
10. White, H. L., Rolf, D., *Am. J. Physiol.*, 1956, v185, 152.
11. Schwarz, K., *Ann. N. Y. Acad. Sci.*, 1954, v57, 878.
12. Mackenzie, C. G., in *Symposium on Nutrition*, R. M. Herriott, edit., Johns Hopkins Press, Baltimore, 1953, p136.
13. Burr, L. H., McLennan, H., *Can. J. Biochem. Physiol.*, 1960, v38, 829.
14. Olson, R., *Ann. Rev. Biochem.*, 1959, v28, 486.

Received October 26, 1962. P.S.E.B.M., 1963, v112.

Mammalian Cell Cultures Contaminated with PPLO III. Elimination of PPLO with Specific Antiserum.* (27985)

MARY E. POLLOCK AND GEORGE E. KENNY† (Introduced by L. C. McLaren)
Department of Microbiology, University of Minnesota, Minneapolis

The discovery in 1956 by Robinson and coworkers that mycoplasma species (pleuropneumonia-like organisms or PPLO) occurred as contaminants of mammalian cell cultures (1) stimulated further investigations of the extent of the problem, and led to a search for means of control which would not incur damage to cells of host cultures. Some broad-spectrum antibiotics have proven effective(2, 3); although previous reports indicated that doses toxic to mammalian cells were necessary, Carski and Shepard have reported elimination of PPLO from cell lines cultivated through 4 successive passages in the presence of only 2.5 μg of tetracycline per ml of medium(3). In this laboratory it was demonstrated that PPLO could be eliminated consistently from monolayer mammalian cell cultures by treatment with non-toxic levels of kanamycin when care was taken to use young, sparsely populated cultures and to allow contact with the antibiotic on all surfaces of the vessels(4). Even though antibiotics may be used simply and effectively, emergence of resistant strains must be anticipated whenever large numbers of microorganisms are concerned, and it is readily apparent that some alternative procedure should be available. With this in mind a study has been carried out to determine whether antiserum specific for the PPLO contaminant of a cell line would, when incorporated into the culture medium, cause eradication of the PPLO. Added impetus was given to this effort when a suspension of cells submitted from another laboratory was found to

* This investigation was supported by grants to Jerome T. Syverton from National Foundation and Nat. Cancer Inst.

† Present address: Dept. of Preventive Medicine, Univ. of Washington, Seattle.