radioactivity of the precipitate is thought to represent undegraded insulin(7), the conclusion is again justified that organ extracts (including in this case diaphragm) from nephrotic animals inactivate insulin less well than such extracts from normal animals.

It is suggested that the smaller amount or activity of a heat-labile insulin-degrading substance ("insulinase"?)(9) contained in the tissues of nephrotic as compared with normal rats may account for the observed(6) longer persistence in the circulation of I.V. injected I^{131} insulin in rats with renal disease.

Summary. 1. The insulin-degrading capacity of rat kidney, liver and diaphragm extracts was determined by incubating them with (a) crystalline insulin and observing blood sugar curves in intact rats following intravenous injection of the mixture and (b) I^{131} insulin and measuring undegraded insulin as represented by TCA precipitable radioactivity. 2. By both measurements, kidney and liver extracts from nephrotic rats were less potent than normal organ extracts in the inactivation of insulin.

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Received October 19, 1962. P.S.E.B.M., 1963, v112.

Distribution of Polyoma Virus Antibodies in Japan. (27983)

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Polyoma virus has been isolated from leukemic mice. It has been found to infect exposed animals very readily, especially those housed in the same room with the leukemic mice(1). Some American mice have been imported into Japan, and there is a strong possibility that polyoma virus has been brought into Japan in them. We have surveyed 12 widely separated mouse colonies in Japan for the presence of polyoma virus antibodies.

Materials and methods. For the present study, mice imported from the United States or Germany, as well as some Japanese mice, were obtained from 12 mouse colonies in Japan (Table I). The study involved the imported strains A, AKR, cb, CBA, C_{57}BL, C_3H, C_3HxDD, DD, DM, Hr^{rh}, R_{III}, RF, Swiss/albino, Swiss/Hauschka, and Zb, and the Japanese strains D_{103} and DDO. On arrival at the laboratory, mice were bled by heart puncture. The serum was heated at 56° C for 30 minutes immediately before use in hemagglutination inhibition tests.

The antigen was polyoma virus strain 2609 from Dr. S. Stewart, and was grown in tissue culture. Pregnant Swiss mice were sacrificed with ether, and the entire embryos were removed aseptically, minced with scissors, and then subjected to trypsinization. After centrifugation, the cells were suspended in an LH medium containing 10% pooled calf serum and small amounts of penicillin and streptomycin. The resulting suspension was transferred to tissue culture bottles and incubated at 36°C. A few days later, when confluent cell sheets had developed, the cultures were inoculated with the virus. As soon as early cytopathogenic changes were observed, the fluids were collected and were assayed for hemagglutinin content with a standard pool of mouse serum. The HA titers of the virus used for this study were 1/254 or higher.

Hemagglutination inhibition tests were performed in the cold. Dilutions of serum, antigen, and cells were made with a pH 7.2 phosphate-buffered saline solution, and 0.25 ml of the diluted serum was mixed with 0.25 ml of the diluted virus, which contained 16 hemagglutinating units. The serum-virus mixture was incubated at 36°C for 1 hour, at the end of which time 0.5 ml of an 0.5% guinea pig erythrocyte suspension was added. After agitation, the test tubes were placed in a cold room (2-4° C), where hemagglutination was observed from time to time. Sera were tested at dilutions of 1/100 and 1/200, and those inhibiting hemagglutination were titrated again repeatedly, to determine the exact hemagglutination-inhibiting activity.

Results are shown in Table II. Of the 12

TABLE I. Location of Mouse Colonies in Japan and Relationship to Polyoma Virus Studies.

Location	Mouse-colony	Polyoma virus studies Begun	Note
Fukuoka	1. Kyushu Univ.	None	
Mishima	2. Mishima Nat. Inst. of Genetics	"	
Nagoya	3. Nagoya Univ., Pathology Dept. I	"	*
"	4. " " " " II	"	"
"	5. " " Surgery Dept.	May 1960	†
Nara	6. Nara Prefectural Med. Sch.	None	‡
Okayama	7. Okayama Med. Sch.	"	
Osaka	8. Osaka Univ. Dental Sch.	Fall 1960	§
"	9. " " Microbiological Inst.	Spring 1961	‖
Sapporo	10. Hokkaido Univ.	None	¶
"	11. Sapporo Med. Sch.	End of 1960	
Sendai	12. Tohoku Univ.	None	

* No connection with polyoma virus studies in Surgery Dept.

† Polyoma virus studies in one room; breeding mice for those studies in next room, cared for by same personnel.

‡ Molony virus studies in progress, however.

§ In another room on the same floor as the mouse colony.

‖ In a room on another floor than the mouse colony. Microbiological Inst. about 200 meters away from Dental Sch.

¶ No connection with mouse colony at Sapporo Med. Sch., 3 km. away.

TABLE II. Incidence* of HI Antibody in Mice by Strain and Colony.

Strain	Colony† 1	2	3	4	5	6	7	8	9	10	11	12
A		0/4	0/23			0/3‡	0/5			2/3		
AKR		0/7						0/6				
cb							0/5					
CBA		0/5								3/3		
$C_{57}BL$		0/4				0/8	0/5					
C_3H	0/19		0/46§				0/10	0/13	0/10	3/3		0/10
C_3HxDD												0/20¶
D_{103}							0/4					
DD						0/8		0/9		0/3		
DDO									0/8			
DM										0/6		
Hr^{rh}										3/5		
R_{III}							0/5					
RF		0/5										
SA		0/23	0/12	0/6							0/5	
SH					0/5							
Zb							0/3					
Misc			0/21									
Total	0/19	0/48	0/102	0/6	0/5	0/19	0/37	0/28	0/18	11/23	0/5	0/30

* Expressed as a fraction: numerator is number of mice with HI antibody, and denominator is number of mice tested.

† Identification Nos. as in Table I.

‡ Offspring of these mice were injected with Molony virus.

§ One of these mice had a liver tumor.

¶ One of these mice had a spontaneous mammary tumor.

TABLE III. Year of Establishment of Imported Strains in Japanese Mouse Colonies.

Strain	Colony* 1	2	3	4	5	6	7	8	9	10	11	12
A		1952	1952			1958	1958			1952		
AKR		1959						1958				
cb							1958					
CBA		1953								1953		
$C_{57}BL$		1953				1956	1958					
C_3H	1956		1956				1952	1958	1956	1952		1956
C_3HxDD												1956
DD						1920		1920		1920		
DM										†		
Hr^{rh}										1958		
R_{III}							1958					
RF		1959										
SA		1953	1958	1953							†	
SH					1960							
Zb							1958					
Misc			†									

* Identification Nos. as in Table I.

† The year of establishment of this strain in this colony could not be ascertained in time for inclusion here.

mouse colonies surveyed, one—that at Hokkaido University—was found to be infected with polyoma virus, about half of the tested mice from the colony (11/23) giving positive tests. In this colony, the A, CBA, C_3H, and Hr^{rh} strains were found to be infected, but not the DD and DM strains. The 4 infected strains all came from the United States originally, and were obtained through the Mishima Nat. Inst. of Genetics. The A and CBA strains at Mishima are HI negative, however, even though the A strains at Hokkaido and Mishima have common ancestors, and likewise the CBA strains. Latent infection is thus a possibility.

The A and C_3H strains originally came from the laboratory of W. E. Heston in 1952, the CBA strain from the City of Hope Medical Center in 1953, and the Hr^{rh} from the Jackson Memorial Laboratory in 1958 (Table III). In 1959, Heston's laboratory and the Jackson Memorial Laboratory were both found by Rowe(2) to be infected with polyoma virus. The other 2 mouse strains at Hokkaido, namely DM and DD, were originally from Germany, and were not infected with polyoma virus.

The inbred mice from the other 10 colonies in Japan came from Houston, the Jackson Memorial Laboratory, Heston or Jay's laboratory, the Roswell Park Memorial Institute, or the City of Hope Medical Center. (The years are given in Table III.) The mice of the Jay-Poily laboratory were negative in 1959(2). Our tests on mice from the 10 Japanese colonies all gave negative results.

The HI-positive sera, of which there were 11, were titrated for HI antibody. Of these 11, 10 had titers of 1:1600 or higher. There was some tendency for older mice to have higher HI antibody titers.

Discussion. Polyoma virus infects not only mice, but also other animals, such as rats and hamsters. It produces kidney sarcoma and subcutaneous tumors in rats, sarcoma and hemangioma in hamsters, and more than 23 kinds of tumors in mice. This virus is highly contagious among these animals.

Rowe has surveyed mice in the United States for the prevalence of antibodies to polyoma virus. His surveys have included mouse colonies in New York City, Bethesda, and Bar Harbor(2), and wild mice in New York City(3). He found such an antibody in most of the 8 colonies surveyed, especially those at institutions where studies involving polyoma virus were being performed. The highest rates of infection are seen when mice are bred near infected animals or the newborn are raised in the same room with them. Older animals become infected much less frequently.

Experimentation with pure cultures of polyoma virus has been started in Japan only very recently, and the possibility of infection from such cultures is negligible. Investigating the possibility of polyoma virus infection through

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.

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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)

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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