antigens are one in the same substance.

Summary. The ability of 2 polyoma virus strains, 3049 and lp-D, to produce transplant immunity in adult hamsters against tumor cells induced by both strains was studied. The 3049 virus immunized hamsters against tumor cells produced by itself, but tended to enhance the growth of lp-D virus-induced tumor cells. On the other hand, the lp-D virus produced little or no immunity against its own tumor cell, but enhanced the growth of 3049 virus-induced cells. X-irradiated tumor cells induced by the 3049 virus, when given to adult hamsters, enhanced the growth of both 3049 and lp-D tumors. Possible explanations for these paradoxical results, based on the presence of at least 2 transplant antigens, were discussed.

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Fatty Acid Metabolism by *in vivo* Labeled Adipose Tissue in Essential Fatty Acid Deficient Rats.* (29925)

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In a previous study it was shown that epididymal fat pads of rats esterify and metabolize palmitic and linoleic acid at a similar rate(1). These results were obtained in normally fed, growing rats, in which the epididymal fat pads were labeled selectively with radioactive fatty acids, using the *in vivo* incubation technique(2). The present communication deals with the comparative uptake and dissimilation of palmitic and linoleic acid in epididymal fat pads of essential fatty acid (EFA) deficient rats.

Materials and methods. Male albino rats of the Hebrew University strain were kept on a fat free diet(3) from weaning. Since hydrogenated oil was not added to the diet the animals were rendered EFA and fat deficient. After 90 days on this diet the linoleic acid content of the epididymal fat pads determined by gas-liquid chromatography(4) fell to 0.5-2.0%, while in control pads 18-22% was found. At this time, *in vivo* incubation of epididymal fat pads, exteriorized with their vascular and neural connections intact, was carried out as described previously(2). The incubation medium (2 ml) consisted of Krebs-Ringer phosphate buffer (Ca^{++} omitted), pH 7.45, with 5 mM glucose, 0.7 mM dialyzed bovine serum albumin (Pentex, Inc.) and 0.7 mM labeled fatty acid. In mixtures of palmitic and linoleic acids equimolar concentrations were used. Incubation time was 20 minutes at 37°. Following incubation the pads were washed(2) and either both pads were removed or one

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TABLE I. Uptake of Palmitic and Linoleic Acid by Epididymal Fat Pads of EFA Deficient Rats During Incubation *in vivo*.

Fatty acids in incubation medium	No. of rats	Wt, g range		mμ moles of total fatty acid incorporated per pad (mean ± S.E.)
		Rat	Pad	
H^3-palmitic + C^{14}-palmitic	21	125-168	.6-1.0	61.32 ± 4.03
H^3-palmitic + C^{14}-linoleic	22	128-152	.6-1.0	65.01 ± 4.04

TABLE II. Comparative Incorporation of H^3 and C^{14} Labeled Pairs of Fatty Acids by the Same Epididymal Fat Pad During Incubation *in vivo*.

Pairs of labeled fatty acids in medium	No. of rats	Ratio of H^3/C^{14} in		H^3/C^{14} in pad different from 1.000†	
		Medium	Pad	t	p
H^3-palmitic + C^{14}-linoleic	22	1.000	1.169*	5.08	<.001
H^3-palmitic + C^{14}-palmitic	21	1.000	.976	1.69	>.1

* Mean.

† The statistical significance was determined by the method of paired comparisons(6) comparing H^3/C^{14} in the pad to that of the medium in the same experiment.

pad was taken out and the other pad was replaced into the abdominal cavity to be examined at later time intervals. Radioactivity was determined on ethanol:ethyl ether (3:1 v/v) extracts of epididymal fat pad homogenates using the Tricarb liquid scintillation spectrometer adjusted to count H^3 and C^{14} simultaneously as described previously(4).

The analysis of labeled products was carried out on thin layer silicic acid chromatoplates, developed in petroleum ether:ethyl ether:glacial acetic acid (72:28:0.6 v/v). 1-C^{14} linoleic acid, 1-C^{14} palmitic acid and 9,10-H^3 palmitic acid were obtained from the Radiochemical Center, Amersham, Great Britain. Using gas liquid chromatography 99, 98 and 97% of the fatty acids were found under the respective peaks; radiochemical purity was checked according to Karmen *et al*(5) and 98.5, 98 and 97% of the radioactivity was recovered in the fatty acid peak, respectively. Non-radioactive linoleic and palmitic acid were obtained from the Hormel Institute, Austin, Minn.

Results. Comparative uptake of palmitic and linoleic acid. Pads of animals with matched body weights were incubated in a mixture of either 9,10-H^3 palmitic and 1-C^{14} linoleic acid or 9,10-H^3 palmitic and 1-C^{14} palmitic acid. It can be seen (Table I) that the total amount of fatty acid taken up by the pads was the same in the presence or absence of linoleic acid in the incubation medium. The comparison of the ratio H^3/C^{14} in each individual pad to that in its incubation medium shows that there was a slight but statistically significant rise in this ratio, indicating that more palmitic than linoleic acid was taken up by the pad (Table II). When 9,10-H^3 palmitic acid and 1-C^{14} palmitic acid served as substrate the ratio of H^3/C^{14} in the pad was the same as in the incubation medium, indicating that the adipose tissue did not discriminate between a C^{14} and H^3 label.

Comparative disappearance of palmitic and linoleic acid. The aim of this experiment was to determine whether in animals depleted in linoleic acid the labeled linoleic acid will be metabolized at a different rate than the simultaneously introduced palmitic acid. Both pads of each animal were incubated with a mixture of either 9,10-H^3 palmitic acid and 1-C^{14} linoleic acid or 9,10-H^3 palmitic acid and 1-C^{14} palmitic acid. After 20 minutes of incubation pad I was removed while pad II was replaced into the abdominal cavity and taken out 30 or 47 days thereafter, during which time the animals continued to be kept on the fat free diet. The radioactivity recovered in pad II, 47 days after incubation, amounted to one-third of that found in pad I of the same animal taken out after 20 minutes of incubation (Table III). The disappearance of the label from pad II indicates that the replaced pad remained metabolically active(1,2). The results in Table III are given as ratios of H^3/C^{14} in pad I and pad

TABLE III. Comparative Disappearance of H^3 and C^{14} Labeled Pairs of Fatty Acids from Epididymal Fat Pads of the Same Rat, Following Incubation *in vivo*.

Pairs of labeled fatty acids in medium	No. of rats	Pad No.	Time*	H^3-radioactivity cpm/pad $\times 10^{-3}$ (mean $\pm$ S.E.)‡	H^3/C^{14} in pad	H^3/C^{14} in Pad II different from 1.000† t	p
H^3-palmitic + C^{14}-linoleic	6	I	20 min	56.7 ± 4.2	1.000§	1.25	>.1
		II	30 days	22.8 ± 4.4	1.023		
H^3-palmitic + C^{14}-linoleic	6	I	20 min	62.6 ± 4.8	1.000	1.63	>.1
		II	47 days	20.6 ± 4.8	.974		
H^3-palmitic + C^{14}-palmitic	6	I	20 min	57.9 ± 1.0	1.000	.48	>.1
		II	47 days	19.3 ± 2.8	1.009		

* Radioactivity in Pad I was determined at the end of 20 minutes of incubation; in Pad II, 30 or 47 days thereafter.

† Statistical significance was determined by the method of paired comparisons(6) comparing Pad II to Pad I of the same animal.

‡ Since the H^3/C^{14} ratio in the pads did not vary from 1.000 the changes in radioactivity content of only one isotope are given.

§ Mean.

II of the same animal and it can be seen that this ratio in pad II did not vary significantly from that in pad I. The lack of change in the H^3/C^{14} ratio in pad II, relative to pad I demonstrates that the 2 fatty acids were dissimilated from the pad at the same rate. Animals in which both pads were labeled with a mixture of H^3 palmitic and C^{14} palmitic acid served as controls. Since after 47 days the ratio of H^3/C^{14} in pad II did not vary significantly from that found in pad I it seems that H^3 palmitic acid can serve as a reliable marker for the above-described comparative study.

Separation of labeled products. The radioactive lipids formed after *in vivo* incubation of the epididymal fat pads were separated on thin layer silicic acid chromatoplates and the results of 4 experiments in which 1-C^{14} linoleic acid served as label are seen in Table IV. In both pads 98% of the radioactivity was recovered in esterified form; significantly more labeled diglycerides and phospholipids were found in pad I than in pad II, removed 30 days later. Similar results were obtained with labeled palmitic acid.

Discussion. The present results are in close agreement with those obtained with animals fed Purina laboratory chow diet in respect to rate of uptake of both fatty acids and their comparative disappearance from the epididymal fat pads(1). Adipose tissue of EFA deficient rats, like that of normal rats does not esterify preferentially an essential over a non-essential fatty acid. Ninety-five per cent of the fatty acid taken up from the medium is incorporated into glycerides, which can be regarded as storage lipids. The essentiality of linoleic acid, the precursor of arachidonic acid, is linked most probably with the latter's presence in structural phospholipids, especially those of mitochondria. In the adipose tissue the cytoplasm forms but a very small part of the total mass of the cell and hence the relative amounts of neutral fat and phospholipids are much in favor of the first. In the rat heart, in which phospholipids amount to 80% of the total lipids, an in-

TABLE IV. Separation of Radioactive Lipids Synthesized After Incubation *in vivo* of Epididymal Fat Pads with 1-C^{14}-Linoleic Acid.

	% of radioactivity							
Labeled product	Pad I (20 min)				Pad II (30 days)			
Rat	1	2	3	4	1	2	3	4
Triglyceride	71.8	68.3	75.8	72.8	96.2	97.0	97.6	95.9
Diglyceride	21.9	23.3	16.7	19.5	1.2	.9	.7	.9
Free fatty acid	1.2	1.4	1.4	1.3	1.4	1.0	.7	1.2
Phospholipid*	5.1	7.0	6.1	6.4	1.2	1.1	1.0	2.0

* Contains traces of monoglyceride.

creased incorporation of labeled linoleic acid into phospholipids was found in EFA deficient animals(7).

In normal, growing rats similar half lives of palmitic and linoleic acids in the adipose tissue were found(1). The estimation of half lives of fatty acids in the selectively labeled adipose tissue of EFA deficient rats was difficult owing to irregular loss of body weight, accompanied by rapid mobilization of the labeled fatty acids from the pads. However, using the 2 isotopic labels the rate of disappearance of linoleic acid in relation to that of palmitic acid in the same pad could be determined and neither conservation of the essential fatty acid nor an increased rate of its removal from the depot was encountered. This is in accord with the findings of oxidation of linoleic acid in EFA deficient mice in which no conservation of the essential fatty acid was reported(8).

Summary. *In vivo* incubated epididymal fat pads of EFA deficient rats were labeled selectively with radioactive palmitic and linoleic acid. The comparative uptake of both fatty acids by the same pad and their dissimilation from the contralateral pad during 47 days after labeling were determined. No preferential uptake or disappearance of linoleic acid in the EFA deficient adipose tissue was found.

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Post-Heparin Lipolytic Activity Following Intravenous Heparin-S^{35}. (29926)

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The relation between circulating heparin and the lipolytic factors which appear in plasma after its injection has been of considerable interest since the original observation of post-heparin clearing(1). Recent studies in rats using relatively high doses of S^{35}-labelled heparin (200 units/kg or about 2 mg/kg) have suggested that the appearance and decay of post-heparin lipolytic activity is parallel to radioactive heparin disappearance (2), raising the possibility of a physicochemical relationship between circulating heparin and the lipolytic enzyme. Other studies in rabbits and man using much lower doses of nonradioactive heparin (0.1 mg/kg)(3) have suggested that heparin rapidly leaves the circulation and does not circulate in significant quantities during the decline in plasma levels of the lipolytic enzyme. This dissociation of heparin and lipolytic activity would make a combination of heparin with the lipolytic enzyme highly unlikely. On the other hand rapid heparin disappearance would allow a quantitative determination of the relationship between the heparin injected and the lipolytic activity appearing in plasma(3). The present study was designed to test the validity of this latter observation by using

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