in vitro for one requiring time-consuming, uncertain, and expensive maintenance in infected rabbits.

Summary. Fluorescent treponemal antibody tests (RFTA and FTA II) using hypochlorite-treated Reiter and Nichols strain treponemes, respectively, were compared with each other and with a Reiter protein complement-fixation (RPCF) test in a study of sera previously subjected to the *Treponema pallidum* immobilization (TPI) test. The fluorescent antibody (FA) tests were closely comparable in sensitivity and specificity for syphilis. The RPCF test was slightly more sensitive than either FA test, but yielded somewhat more "non-specific" reactions. It appeared that either FA test might serve as well as the RPCF test as a screening procedure in differentiating persons with latent syphilis from those whose sera react "non-specifically" with cardiolipin antigens. Considerations favoring selection of an FA test are simplicity of performance and the need for fewer reagents. Moreover, the RFTA test substitutes an organism that can be cultivated simply *in vitro* for one requiring maintenance in infected rabbits.

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Effects of Mannoheptulose on Glucose Metabolism of Isolated Tissues. (27277)

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Parenteral administration of mannoheptulose produces hyperglycemia and hyperketonemia in rats(1,2). It has been suggested

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that mannoheptulose produces these effects by blocking the release of insulin from the pancreas(2). However, the possibility of a direct action of mannoheptulose on glucose metabolism in major tissues was not ruled out. This report deals with effects of mannoheptulose on the metabolism of glucose by isolated tissues.

Methods. Sprague-Dawley rats were fed, *ad libitum,* a stock diet (Purina chow). All were in the fed state when killed by cervical fracture. Slices of liver and kidney (largely cortex) were prepared freehand, blotted free of excess moisture and weighed. About 300 mg were incubated in 3.2 ml of Krebs bicarbonate buffer(3). Glucose, unformly labeled with C^{14}, was present at an initial concentration of 1.3 or 6.2 μM/ml. Mannoheptulose† was added dissolved in buffer. The utilization of glucose during the incubation (37.2° for 1 hour) was determined by the disappearance of C^{14}-glucose from the media and its presence as $C^{14}O_2$ and lipid-C^{14}. Since the dilution of the C^{14}-glucose by the glucose of the slices was not affected by mannoheptulose, the ratio of C^{14} values to total amounts of glucose used is the same for paired experiments.

Diaphragms were obtained from rats weighing 150-200 g. Hemidiaphragms were incubated (2 hours at 37.2°) in 2.2 ml of media containing either glucose or glucose and mannoheptulose.

Epididymal fat pads were divided into 4 equal parts of 250-300 mg each. The effect of mannoheptulose on glucose metabolism was determined in the presence and absence of crystalline insulin.‡

Measurement of radioactivity. Incubations were carried out in 25 ml Erlenmeyer flasks. When CO_2 was collected the flask contained a well hanging from a serum stopper. At the end of the incubation period, 0.2 ml of 1N NaOH was added to the well and 0.2 ml of 5N H_2SO_4 to the main compartment of the flask(4).

† Crystalline mannoheptulose(1) was prepared from avocados at Weizmann Inst., Rehovoth, Israel.

‡ Obtained from Dr. Mary Root, Eli Lilly Labs., Indianapolis, Ind.

TABLE I. Effect of Mannoheptulose on Glucose Uptake by Slices of Liver and Kidney.

	Initial media†		Added C^{14} glucose‡		
	Glucose	Manno-heptulose	Dis-appeared	Recovered as CO_2	Recovered as Lipid
Tissue*	mM		μM/g tissue/hr		
Liver	1.3	—	.9	.2	.02
	1.3	4.5	.6	.1	.02
	6.2	—	8.9	.3	.06
	6.2	5.0	9.0	.2	.06
Kidney	1.3	—	3.4	.9	.05
	1.3	4.5	3.4	1.1	.05

* Female rats weighed 200-250 g. Tissue slices prepared freehand; for liver 200-300 and kidney 150-300 mg/flask.

† 3.2 ml Krebs bicarbonate solution/flask. Incubated for 1 hr at 37.2°.

‡ Calculated on basis of specific activity of the C^{14}-glucose before addition of tissue.

Lipids were isolated by extraction with 2:1 chloroform-methanol(5). Aliquots of chloroform extract were hydrolyzed by heating with alcoholic KOH, acidified and fatty acids extracted with Dole's solution(6).

The radioactivity of CO_2, lipid and aqueous fractions were determined by the method of Bray(7) with an automatic Packard Tri-Carb Liquid Scintillation Spectrometer. The C^{14} values of the fatty acids were subtracted from those for total lipids to yield the radioactivity of the glyceride "glycerol."

Glucose was determined by means of glu-

TABLE II. Effect of Mannoheptulose on Glucose Uptake by Rat Diaphragm.

		Initial media†		
		Glucose	Manno-heptulose	Glucose uptake,‡
Rat #*	Sex	mM		μM/g tissue/hr
4	♂	5.7	—	8.1
		5.7	13.7	8.9
5§	♂	5.7	—	17.9
		5.7	13.7	15.5
6	♀	5.3	—	8.4
		5.3	13.7	5.9
7	♀	5.3	—	9.8
		5.3	13.7	12.8

Avg ratio for paired flasks: 1.11 ± 0.09 (7 exp.)

* Rats weighed 150-200 g.

† 2.2 ml of Krebs bicarbonate solution/flask; gas phase 95% O_2 - 5% CO_2; incubated 2 hr at 37.2°.

‡ Calculated from difference between initial and final values.

§ Insulin added (55 milliunits/ml).

TABLE III. Effect of Mannoheptulose on Glucose Metabolism of Epididymal Fat Pad.

Initial media concentration*				C^{14} glucose recovered as		
Glucose	Manno-heptulose	Insulin,†	Glucose uptake,	Total lipid	Fatty acids	Glyceride glycerol
mM		units/l	μM/g/hr	μM added glucose/g/hr		
5.3	—	—	1.7 ± .4‡	.77 ± .03	.37 ± .05	.40 ± .03
5.3	13.7	—	2.4 ± 1.0	.79 ± .07	.38 ± .07	.41 ± .03
5.3	—	55	8.9 ± 2.0	5.5 ± 2.0	4.2 ± 1.7	1.2 ± .2
5.3	13.7	55	7.0 ± 1.5	4.5 ± 1.4	3.4 ± 1.2	1.2 ± .2

* 2.2 ml Krebs bicarbonate solution/flask; gas phase 95% O_2 - 5% CO_2; incubated 2 hr at 37.2°.

† Glucagon-free crystalline Zn insulin.

‡ Mean ± stand. error. Results of 3 rats.

cose oxidase (Worthington Biochemical Corp., Freehold, N. J.).

Results. Liver and kidney slices (Table I). Mannoheptulose did not alter the disappearance of C^{14}-glucose from the medium nor its recovery as $C^{14}O_2$ or lipid-C^{14} even when the ratio of mannoheptulose to glucose was greater than 3. In addition, the fraction of the lipid-C^{14} in fatty acids was found to be unaffected by the presence of mannoheptulose.

Diaphragm (Table II). The glucose uptake by hemidiaphragms was the same in the presence of mannoheptulose as in its absence. Increasing the molar ratio of mannoheptulose to glucose from about 1 to more than 3 did not appreciably alter glucose uptake. In representative tests the recovery of the glucose-C^{14} as CO_2 and fatty acids was not altered by the heptulose. The action of insulin in stimulating glucose uptake was not affected appreciably by mannoheptulose (rat 5, Table II).

Epididymal fat pat (Table III). Addition of mannoheptulose to the medium did not inhibit the disappearance of glucose nor recovery of C^{14} in the lipid fraction. The distribution of lipid-C^{14} between fatty acids and glyceride "glycerol" was about equal in both sets of flasks. When insulin was added to the medium, glucose uptake and lipid-C^{14} increased to the same degree whether or not mannoheptulose was present. The insulin-stimulated increase in fatty acid-C^{14} was about 10-fold while that in glucose uptake and glyceride "glycerol-C^{14}" was only 3- to 5-fold.

Discussion. The diabetogenic action of mannoheptulose could be due to its effects on: 1) the carbohydrate metabolism of insulin sensitive tissues (*e.g.*, the action of 2-deoxyglucose(8)), 2) insulin, *per se* (*e.g.*, anti-insulin(9)); or 3) the production and secretion of insulin from the pancreas (*e.g.*, after alloxan injection).

The present investigation shows that mannoheptulose does not inhibit uptake and metabolism of glucose by liver, kidney, adipose tissue or diaphragm. The stimulatory action of insulin on the carbohydrate metabolism of diaphragm and adipose tissue, also, is unaffected by mannoheptulose, confirming the earlier observation(2) that mannoheptulose has no effect on the action of exogenous insulin in fasting rats. In addition, blood glucose and ketone body levels of pancreatectomized rats were not changed by mannoheptulose. It was suggested(2) therefore, that mannoheptulose may act in the pancreas by suppressing release of insulin. This suggestion is supported by the results presented here.

Summary. The mechanism of the diabetogenic action of mannoheptulose has been investigated by testing its action on glucose metabolism by isolated tissues. Mannoheptulose did not alter the *in vitro* metabolism of glucose by liver, kidney, diaphragm or epididymal fat pad. The stimulating effects of insulin on glucose metabolism of diaphragm and adipose tissue were not decreased by mannoheptulose.

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Homotransplantation Immunity of Neonatal Rabbits.* (27278)

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The development of the immune response in neonatal animals has been the subject of many investigations(1). Most of these studies have dealt with the humoral antibody responses of neonates injected with soluble protein or bacterial antigens and have revealed little or no antibody formation. At the same time in the neonatal period, homograft immunity is apparently already developed, but detailed information concerning the degree of development is not available for most species. More precise knowledge of the status of homograft immunity in the neonatal rabbit is of importance in interpreting experiments in which the immunologic behavior of adult homologous lymphoid cells, transferred to neonatal recipients, has been observed. In some of these experiments, adult cells transferred to neonates have made little or no antibody (2-5). One possible explanation for this failure has been the homograft reaction by the neonatal recipient against the transferred cells(6).

It was the purpose of this study to determine the transplantation immunity of the neonatal rabbits as measured by rejection of skin homografts. In addition, the effect of prior irradiation on survival times of homologous skin grafts was also observed.

Method. Pregnant albino rabbits were obtained from several different breeders and after delivery the nursing neonates were used as donors and recipients of skin grafts. Neonatal skin grafts were not exchanged between litter mates. A total of 172 neonates were used in this study and were divided into 2 major groups for skin grafting. The first group of animals was grafted within the first week of life and ranged in age between 2 and 6 days. The second group consisted of animals 10 to 14 days old. One-half the members of each group of neonates received adult homologous skin, the other half received neonatal homologous skin.

Thirty-three adult non-pregnant rabbits weighing between 2.5 to 3.5 kg were obtained from the same breeders and served as both skin graft donors for the neonates and as control recipients for homologous adult and neonatal skin grafts. Approximately one-half of the neonatal and adult recipients received 400 r total body irradiation 48 hours prior to skin grafting(7).

Full thickness skin grafts were taken from the dorsum of the ear of all neonates. The adult donor skin grafts were full thickness grafts from the dorsum of the ear or split thickness grafts from the abdominal skin. Split thickness grafts were used in order to arrive at a more comparable physical quantity to neonatal skin. All the skin grafts were fitted and sutured to the dorsum of the ear of the recipients and measured 0.5 × 0.5 cm in neonates and 1 × 1 cm in adults. In approximately one-half of the recipient neonates, the skin removed to create the defect

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