fection in the cow. Within the limits of this study, the considerable differences in rates of inactivation also permit one to distinguish both types of nonspecific agglutinins as well as the specific agglutinin by use of a combination of both 56°C and 65°C heat inactivation tests.

The extremes in the range of rates of heat inactivation of specific agglutinins from the 9 cows studied, happened to be represented by one cow with a 12-month history of infection (least stable) and another cow with a known infection of the order of 6 weeks (most stable). The number of sera involved precludes any interpretation of this observation.

Summary. Two types of nonspecific agglutinins for Brucella were distinguished through kinetic studies of their inactivation by heat. Rates of inactivation of specific and nonspecific agglutinins differ to an extent that can be accounted for only by a difference in molecular species. Heat inactivation of nonspecific and specific agglutinins for Brucella is a first order reaction.

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Metabolism of Specifically Labelled Glucose in Adipose Tissue from Alloxan-Diabetic Rats.* (27272)

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Metabolic studies with adipose tissue *in vitro*, using specifically labelled glucose, have suggested the phosphogluconate oxidation pathway as a major route of glucose metabolism. Milstein(1) comparing the oxidation of glucose-1-C^{14} and glucose-6-C^{14} to CO_2 in rat adipose tissue concluded that this pathway was the principal route of glucose metabolism and was preferentially stimulated by insulin. Likewise, in adipose tissue from alloxan-diabetic animals, the conversion of glucose carbon-1 to CO_2 was found to be proportionally more depressed than was glucose carbon-6 to CO_2, suggesting a selective decrease in this pathway. Winegrad and Renold(2), reporting on the effects of insulin added *in vitro* on the oxidation of specifically labelled glucose to CO_2 in a similar system, corroborated the findings of Milstein. However, they also measured the recovery of labelled carbon in tissue fatty acids and found that although insulin greatly increased the incorporation of both carbon-1 and carbon-6 into fatty acids, the ratio of C1/C6 in fatty acids in both control and insulin-stimulated tissues remained approximately 0.5. Subsequently, in experiments(3,4) in which both concentration of glucose and/or insulin were independently varied, this ratio persisted with all experimental combinations, suggesting that the action of insulin was to accelerate glucose entry into the metabolic pathways and not to stimulate selectively any specific pathway of glycolysis.

The purpose of this report is to extend the above studies to include not only the oxidation of specifically labelled glucose to CO_2

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TABLE I. Comparison of Animal Weights and Lipid/Nitrogen Ratios in Epididymal Adipose Tissue from Normal and Alloxan-Diabetic Rats.

	n	Animal wt (g)	Lipid/nitrogen ratio
Alloxan-diabetic	8	184 ± 13	165 ± 39
Normal	8	301 ± 9	445 ± 14

but also its recovery in fatty acids, glyceride-glycerol and glycogen in adipose tissue from normal and alloxan-diabetic rats.

Materials and methods. Male albino rats of the Wistar strain (Charles River Laboratories) were fed Purina pellets *ad libitum*. Alloxan (40 mg/kg) was administered intravenously to one-half the animals after a 48-hour fast. These animals were used 3-4 weeks after alloxan only if a random blood glucose concentration was greater than 300 mg %. Initial weights of all animals were between 115 and 150 g. The mean of final animal weights are listed in Table I. All animals were exposed to food until 2 to 3 hours before the experiment.

For the experiments, the animals were decapitated, and the epididymal adipose tissue excised and placed in 25 ml Erlenmeyer incubation flasks containing 3 ml Krebs-Ringer bicarbonate buffer with 20 mM (360 mg %) specifically labelled glucose, and shaken at 38° for 3 hours(5). In the normal animals a distal portion of the fat pad weighing 200 to 300 mg was placed in each flask. In the diabetic animals, as much tissue was used as could be excised. The flasks were equilibrated with 95% O_2:5% CO_2 before addition of tissue and for the first 8 minutes of incubation. To increase the recovery of radioactivity in tissue fatty acids from alloxan-diabetic animals, each flask contained 5 μC of glucose-C^{14}, approximately 10-fold the amount used by Winegrad and Renold(5). Precise specific activity was determined by addition of carrier glucose and preparation and recrystallization of the phenylosazone followed by counting on stainless steel planchets in a thin window proportional flow counter. The preparation of tissue derivatives (fatty acids, glyceride-glycerol and glycogen) as well as the isolation of CO_2 and the radioactive determination of these moieties have been described previously(2,4,5). Specifically labelled glucose was purchased from the New England Nuclear Laboratories, Boston, Mass.

Results. In Table I are summarized animal weights and lipid/nitrogen ratios in epididymal adipose tissue excised from normal rats and alloxan-diabetic littermates. The severity of the diabetic state as measured by body weight loss, or failure to gain, was found to parallel the ratio of adipose tissue lipid to adipose tissue nitrogen (Fig. 1).

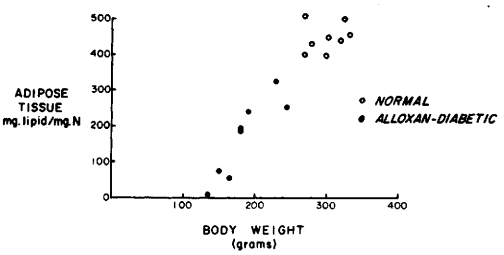

FIG. 1. Comparison of total body wt and adipose tissue lipid/nitrogen ratios in normal and alloxan-diabetic litter mates.

In Table II are summarized data from *in vitro* incubation of this adipose tissue with glucose-1-C^{14} or glucose-6-C^{14} (20 mM). With approximate corrections for nitrogen content and for differences in medium glu-

TABLE II. Metabolism of Glucose-1-C^{14} and Glucose-6-C^{14} in Adipose Tissue from Normal and Alloxan-Diabetic Rats *In Vitro*.

		CO_2	Tissue fatty acids	Glycogen	Glyceride-glycerol
Normal (n = 8)	Carbon-1	1.24 ± .46	.229 ± .051	.018 ± .003	.263 ± .025
	Carbon-6	.47 ± .06	.550 ± .192	.022 ± .006	.479 ± .039
	C-1/C-6	2.7 ± .3	.56 ± .10	1.24 ± .28	.85 ± .20
Alloxan-diabetic (n = 8)	Carbon-1	.63 ± .09	.012 ± .003	.015 ± .002	.368 ± .065
	Carbon-6	.33 ± .04	.022 ± .008	.017 ± .004	.403 ± .085
	C-1/C-6	1.9 ± .2	.60 ± .09	1.05 ± .15	.95 ± .15

Values in μmoles specifically labeled glucose carbon per mg tissue N per 3 hr.
Glucose 20 mM. Krebs-Ringer bicarbonate buffer.

cose concentrations, the values agree well with those previously reported(1,3) and again demonstrate the decreased oxidation to CO_2 of glucose-1-C^{14} and glucose-6-C^{14} in the diabetic with a greater depression of the former. Likewise, the marked depression in fatty acid synthesis, to about 5% of that in the normals, is noted in the diabetic. Surprisingly, however, the C-1/C-6 ratio in fatty acids of 0.5 is not significantly altered. No significant depression in incorporation of glucose carbon is found in glycogen or glyceride-glycerol.

Discussion. Previous studies(2,3,4) have demonstrated a ratio of glucose C-1/C-6 incorporation of approximately 0.5 into fatty acids in adipose tissue excised from normal rats and incubated *in vitro*. Addition of insulin to the incubation medium markedly increased fatty acid synthesis 5- to 20-fold, but the ratio in the fatty acids persisted, suggesting accelerated glucose metabolism without any specific alteration in pathways. Precise quantitation of these pathways, the phosphogluconate oxidative pathway and the Embden-Meyerhof pathway, is impossible with the data from these experiments(6), but the ratio of C-1/C-6 of approximately 0.5 in fatty acids suggests equal metabolism of glucose by each pathway. The experiments in this report add to the previously reported data and indicate that even in the diabetic animal, where fatty acid synthesis is markedly decreased, the pattern of metabolism of glucose is similar, as evidenced by persistence of the C-1/C-6 ratio in fatty acids of close to 0.5.

Lastly, as reported previously(7) the basal incorporation of glucose carbon into glycogen and glyceride-glycerol is not markedly depressed in the diabetic when compared to the normal. Other studies(8,9) have shown that a high concentration of free fatty acids in the medium induces glyceride-glycerol synthesis in adipose tissue, and the elevated level of these free fatty acids in adipose tissue from the diabetic, in accord with their accelerated rate of release, causes a selective incorporation of whatever glucose enters the metabolic pool of the diabetic into glyceride-glycerol.

Summary. Epididymal adipose tissue from normal and alloxan-diabetic rats was incubated *in vitro* with glucose-1-C^{14} and glucose-6-C^{14}. Recovery of labelled carbon in fatty acids showed a marked depression in tissues from diabetic animals, but the ratio of C-1/C-6 in fatty acids showed no change, suggesting a proportional depression of both the glycolytic and phosphogluconate oxidation pathways. These data supplement previous observations that in the presence of insulin both pathways are equally stimulated.

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