about 40% by 0.1 *M* NaCl but not by even higher concentrations of KCl. Probably Na^+, and not an impurity in the NaCl used, produced the inhibition because NaCl from a second source gave the same results, and in addition, rates of hydrolysis when using a buffer made from the mono- and dipotassium phosphates were double those found when using a buffer made from the corresponding sodium phosphates. No evidence was found that Na^+ inhibited, not thrombin, but a contaminant in the 4 thrombin preparations tested. Increasing concentrations of glycerol in the tests produced increasing inhibition of hydrolysis, but the percentage inhibition due to Na^+ remained constant. Incubating thrombin at 37° and pH 3.4 gradually destroyed its ability to hydrolyze TAME, but at all times tested the percentage inhibition due to Na^+ remained constant. SBTI did not inhibit the rates, and the percentage inhibition by Na^+ was the same in the presence and in the absence of SBTI. For these reasons it was concluded that thrombin itself is inhibited by Na^+.

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Growth Hormone, Plasma Glucose, and Ketone Bodies as Determinants of Cardiac Glycogen in Normal and Diabetic Rats* (32713)

ROBERT L. HAZELWOOD
Department of Biology, University of Houston, Houston, Texas 77004

An impressive body of evidence has accumulated indicating that both cardiac metabolism and cardiac contractile activity rely preferentially on noncarbohydrate sources of energy. That fatty acid metabolism is most closely linked with such energy demands has been indicated in many studies carried out on mammalian hearts both at rest and during exercise, employing *in vitro* and *in vivo* techniques (1–6).

Elevated cardiac glycogen levels in rats resulting from feeding free fatty acids (7,8), during fasting (9), and in diabetes (8,10) have been observed; the need for adequate circulating growth hormone for such a polysaccharide increase during fasting has been well defined (9,11). Not all studies, however, have divorced plasma ketone levels from plasma glucose levels as possible influencing factors in regulating cardiac glycogen concentrations. Furthermore, the observation that the diabetic rat hypophysis contains one sixth the normal amount of growth hormone (12, 13) is yet to be reconciled with the elevated heart glycogen levels frequently reported in such animals (10).

It appeared of interest to undertake studies which would attempt to clarify the interrelationships among cardiac glycogen, plasma glucose, and plasma ketone levels. Also attempts were made to assess cardiac polysaccharide responses to exogenous growth hormone in the fasted and nonfasted diabetic rat.

Methods. All rats used were male Cheek-Jones (Houston) rats derived from the Holtzman strain, kept at 75±1°C and fed Purina laboratory chow and water *ad libitum*. Blood and urinary glucose were determined by the Nelson-Somogyi colorimetric method (14), tissue glycogen by the anthrone method (15), and total and differential plasma ketones (details by personal communication) by the method of Bessman and Anderson (16). In this ketone method double oxidation of acetoacetate and β-hydroxybutyric acid by sulfuric acid and dichromate allow for estimation of β-hydroxybutyric acid by subtracting acetoacetate levels from those of total acetone. Plasma protein was determined by Lowry

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TABLE I. Effects of Various Methods of Sacrifice on Tissue Glycogen, Plasma Glucose, and Plasma Ketone Levels.

Method of sacrifice	No. of obser.	Body wt. (gm)	Plasma glucose (mg/100 ml)	Plasma ketones (mg/100 ml)	Tissue glycogen		
					Heart (mg/100 gm)	Liver (%)	Gastrocnemius (mg/100 gm)
Ether	12	229 ± 4.5[a]	143 ± 5.9	2.5 ± 0.3	499 ± 14.3	5.24 ± 0.99	496 ± 12.7
Cervical dislocation	13	232 ± 5.5	115 ± 2.4	2.1 ± 0.4	401 ± 16.2	5.37 ± 0.35	420 ± 9.7
Pentobarbital	12	229 ± 5.2	144 ± 3.0	2.2 ± 0.3	505 ± 10.6	5.25 ± 0.25	526 ± 25.6
Decapitation	13	238 ± 7.2	135 ± 3.8	2.4 ± 0.2	414 ± 12.0	5.65 ± 0.23	537 ± 14.4

[a] Mean ± SE.

et al. (17) and amino nitrogen by the method of Frame *et al.* (18).

Collection of urinary glucose was made from diabetic rats (35 mg alloxan/kg body wt. via tail vein) placed in individual screened bottom metabolic cages employing toluene as a preservative. Growth hormone was a gift of the Endocrinology Study Section, lot no. GH-B9, and when used was injected intraperitoneally twice daily.

Results. It was desirable to establish what effects, if any, various methods of sacrifice had on the major parameters to be studied. Table I presents the effects of four different methods of sacrifice on tissue glycogen, plasma glucose, and total plasma ketone bodies. Plasma ketones and liver glycogen were unaffected. However, struggle in the cervically dislocated rats encouraged greater skeletal muscle glycogenolysis ($p<.01$) and the ruptured vessels in both this type sacrifice as well as in the guillotined rat favored greater loss of cardiac glycogen than in the other two groups ($p<.01$, .01 respectively). The latter has been observed before in our laboratory (6). From these observations it was concluded that sodium pentobarbital was the anesthetic of choice in subsequent studies.

Rats were fasted up to 120 hours and the influence of this regimen on the plasma ketones and glucose and tissue glycogen levels are presented in Table II. The immediate drop in blood glucose and liver glycogen is evident and the subsequent increase in both of these variables as the starvation period was prolonged agrees well with results of many other workers using different animal species. Cardiac glycogen increased significantly ($p<.01$) during the entire fasting period and agrees with observations of Adrouny and Russell (9). Total plasma ketones rose progressively, paralleling the increase in cardiac glycogen ($r = .81$); however, the relative proportion of the various ketone bodies was not altered by the starvation regimen. Failure to observe shifts among the various ketone body levels during fasting has been reported previously (19).

To gain additional insight as to the role which blood glucose or ketone bodies play as determining factors of cardiac glycogen

TABLE II. Effects of Fasting on Plasma Glucose, Ketone Bodies, and Tissue Glycogen Levels in Rats.

Groups	No. of obser.	Body wt.[a] loss (%)	Plasma glucose (mg/100 ml)	Tissue glycogen		Plasma ketone bodies (mg/100 ml)		
				Heart (mg/100 gm)	Liver (%)	Total	Acetoacet.	β-Hydroxy.
Nonfasted controls	10	—	153 ± 6.4	482 ± 9.9	5.08 ± 0.30	3.0 ± 0.3	0.7 ± 0.3	2.3 ± 0.3
1-day fast	12	7.3 ± 0.69[b]	101 ± 1.7	601 ± 15.0	0.19 ± 0.04	10.4 ± 0.9	2.5 ± 0.4	7.9 ± 0.6
2-day fast	10	16.7 ± 0.56	133 ± 4.5	607 ± 13.5	0.22 ± 0.02	12.9 ± 1.3	2.9 ± 0.3	10.0 ± 1.1
3-day fast	13	20.1 ± 0.52	138 ± 4.8	679 ± 12.8	0.32 ± 0.04	12.3 ± 0.6	2.3 ± 0.2	9.9 ± 0.6
5-day fast	13	26.9 ± 0.48	132 ± 4.8	704 ± 10.8	0.41 ± 0.03	18.7 ± 1.8	4.6 ± 0.4	14.1 ± 1.8

[a] Initial body wt. ranged from 228–254 gm.
[b] Mean ± SE.

levels, 98 rats were alloxanized and after a 5-week recovery period were grouped according to severity of diabetes as judged by plasma glucose levels. Once again (Table III) it was found that the best correlation of cardiac glycogen levels was with plasma total ketone levels ($r = .84$) and not with the widely varying plasma glucose levels ($r = .46$). There appeared to be a shift in percentage beta-hydroxybutyrate component to the acetoacetate moiety as a result of the diabetic state; however, the various groups did not vary among themselves in this respect. Liver glycogen levels were consistently below those levels of fed normal rats but well above those levels of 16 or 24-hour fasted intact rats.

An attempt to correlate various parameters with cardiac glycogen in diabetic rats is presented in Fig. 1 where it can be seen that only plasma ketone levels show a high positive correlation ($r = .84$, $p < .001$). It is interesting to note that a moderate positive correlation was found between cardiac glycogen and fasting urine glucose ($r = .65$); Beatty *et al.* observed in alloxanized rats a much closer relationship between ketonuria and glycosuria than between ketonuria and blood glucose (20).

Diabetic rats were fasted for periods up to 72 hours (beyond this period the mortality was extremely high) and the effects observed on plasma and tissue constituents (Table IV). Unlike the normal intact animal, only at 12 hours after initiation of the fasting period was there any indication of increased cardiac glycogen levels ($p = .05$). Plasma total ketones decreased markedly to a value 80% below that of the fed controls after 72 hours whereas plasma glucose decreased by 54% during the same period. Still, after 72 hours of fasting, plasma glucose levels were double those of normal nondiabetic rats; in contrast both the plasma ketone levels and the cardiac glycogen levels of these diabetic rats were at levels considered normal for the nondiabetic animals (compare with Tables I and II). It is of interest to note that fasting does not alter liver glycogen content; these levels remained considerably higher than those of fasting nondiabetic animals. These obser-

TABLE III. Relationship of Plasma Glucose and Ketone Levels with Cardiac Glycogen in the Diabetic Rat.

Diabetic rats grouped by fasting plasma glucose levels[a] (mg/100 ml)	No. of obs.	Body wt. (gm)	Plasma glucose (mg/100 ml)	Plasma ketone bodies (mg/100 ml)			Tissue glycogen		Urine glucose (mg/24 hours)	
				Total	Acetoacet.	β-Hydroxy.	Heart (mg/100 gm)	Liver (%)	Fed	Fast 16 hours
Fed controls	16	168 ± 9.7[b]	647 ± 17.7	13.7 ± 1.3	6.9 ± 1.1	6.8 ± 1.0	747 ± 31.2	2.79 ± 0.3	6869 ± 243	—
Less than 200	10	242 ± 15.5	172 ± 7.6	12.8 ± 3.0	6.1 ± 1.6	7.7 ± 1.6	765 ± 20.4	2.73 ± 0.1	5786 ± 607	2455 ± 316
201–400	16	210 ± 15.2	296 ± 16.7	12.6 ± 1.8	5.2 ± 1.0	7.4 ± 1.0	745 ± 44.2	2.43 ± 0.2	5862 ± 192	3022 ± 163
401–600	42	156 ± 6.1	524 ± 8.3	18.8 ± 0.9	6.7 ± 0.6	12.1 ± 0.8	928 ± 25.6	2.01 ± 0.1	6986 ± 267	4747 ± 213
Above 600	14	139 ± 7.6	634 ± 8.1	18.9 ± 2.3	8.6 ± 1.4	10.5 ± 1.7	926 ± 46.5	1.87 ± 0.1	7389 ± 372	4723 ± 356

[a] All experimental groups were fasted 16 hours prior to sacrifice.
[b] Mean ± SE.

TABLE IV. Influence of Fasting on Plasma Glucose, Plasma Ketone Bodies, and Tissue Glycogen of Alloxan-Diabetic Rats.

Fasting period (hours)	No. of obs.	Final body wt. (gm)	Blood glucose (mg/100 ml)	Plasma ketone bodies (mg/100 ml)			Tissue glycogen		Urinary glucose (mg/24 hours)			
				Total	Acetoacet.	β-Hydroxy.	Heart (mg/100 gm)	Liver (%)	Fed	Fasting period (hours) 6–24	48	72
Fed controls	10	213 ± 26.9[a]	561 ± 10.4	13.12 ± 0.77	3.92 ± 0.26	9.20 ± 0.57	794 ± 29.4	1.90 ± 0.25	8197 ±435	—	—	—
6	10	163 ± 13.2	559 ± 21.6	15.58 ± 1.94	5.56 ± 0.74	10.02 ± 1.28	870 ± 17.0	1.65 ± 0.12	9757 ±322	7062 ±423	—	—
12	9	159 ± 9.6	565 ± 16.1	11.41 ± 2.24	4.21 ± 0.94	7.20 ± 1.54	921 ± 38.0	1.80 ± 0.16	9839 ±511	7902 ±293	—	—
24	9	154 ± 10.4	493 ± 28.4	6.85 ± 1.37	2.24 ± 0.32	4.61 ± 1.06	798 ± 58.7	1.79 ± 0.20	8325 ±562	1369 ±306	—	—
48	9	128 ± 9.2	251 ± 28.1	3.95 ± 0.71	0.85 ± 0.11	2.10 ± 0.73	452 ± 36.3	1.85 ± 0.19	9220 ±382	1503 ±133	720 ±225	—
72	7	124 ± 7.0	259 ± 33.2	2.71 ± 0.44	0.74 ± 0.07	1.97 ± 0.38	427 ± 30.3	1.21 ± 0.25	10,729 ±617	1277 ±198	111 ±41	16 ±2

[a] Mean ± SE.

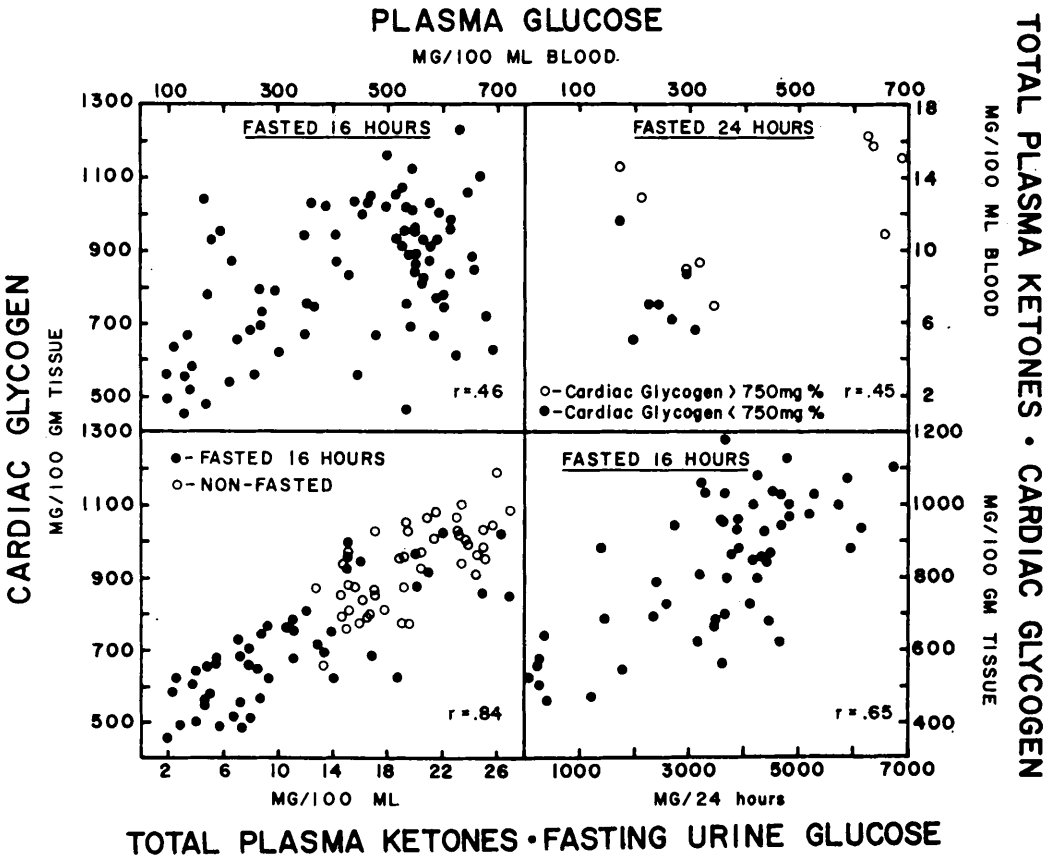

FIG. 1. Relationship of plasma glucose, plasma total ketone bodies, and fasting urine glucose to cardiac glycogen in the diabetic rat. All rats were employed 5–7 weeks after alloxan administration. Each dot represents a single observation.

vations are in good agreement with those of other workers (22,23).

Because of the well-known yet poorly understood interrelationships among fasting, growth hormone, diabetic ketonemia, and cardiac glycogen it was felt desirable to administer growth hormone to fasted and to nonfasted diabetic rats and follow changes in the various plasma and tissue constituents under study. Table V presents results of a study where fasted or nonfasted diabetic rats were injected either with 0.5 mg or 1.5 mg of bovine growth hormone over 1 or 3 days, respectively. Growth hormone promoted small but definite increases in body weight in the nonfasted diabetic rats; also the hormone was effective in diminishing the weight loss due to fasting regardless of total amount administered ($p<.05$ and .01). Growth hormone did not increase cardiac glycogen significantly in nonfasted diabetic rats; actually, the cardiac levels of polysaccharide decreased significantly in the group injected for 3 days ($p<.01$). Superimposing the anabolic hormone on a 24-hour period of fasting depressed cardiac glycogen levels even further ($p<.01$) in the 1-day injected diabetic group, though the 3-day injected group showed the same tendency but was not statistically significant (see below). The decrements in cardiac glycogen were attended by decrements in blood glucose induced by fasting and even more so by the addition of somatotropin. Concomitant significant depressions occurred in total and individual plasma ketone bodies as the result of fasting with or without exogenous somatotropin ($p<.01$ compared with appropriate controls). The imposed regimen

TABLE V. Influence of Exogenous Growth Hormone on Plasma Glucose, Plasma Ketones, and Cardiac Glycogen of Diabetic Rats.[a]

Group	No. of obs.	Body wt. (gm)		Tissue glycogen		Plasma analyses (mg/100 ml of blood)					
		Initial	Change	Heart (mg/100 gm)	Liver (%)	Glucose	Total ketones	Aceto-acet.	β-Hydroxy.	Amino N_2	Protein (%)
Fed 24 hour control rats	11	205 ±13.4[b]	+ 0.4 ± 0.6	976 ±40.6	1.71 ±0.15	622 ±28.5	13.01 ±0.67	3.05 ±0.35	9.96 ±0.70	7.54 ±0.31	7.12 ±0.25
Fed + 0.5 mg GH/24 hours	11	211 ±16.4	+ 4.6 ± 0.5	1058 ±43.4	1.60 ±0.16	640 ±25.8	10.59 ±0.91	2.79 ±0.37	7.80 ±0.67	8.09 ±0.22	7.06 ±0.14
Fasted 24 hours control rats	10	194 ±16.5	−33 ± 1.5	872 ±40.8	2.15 ±0.29	481 ±29.6	8.79 ±0.89	1.78 ±0.16	7.01 ±0.77	8.84 ±0.44	6.78 ±0.34
Fasted 24 hours + 0.5 mg GH	11	259 ±16.4	−27 ± 2.3	648 ±36.2	1.55 ±0.20	220 ±24.9	9.78 ±0.72	1.94 ±0.22	7.84 ±0.74	6.97 ±0.23	9.19 ±0.31
Fed 72 hours control rats	11	219 ±14.7	+ 2.5 ± 0.7	1042 ±40.6	2.15 ±0.24	616 ±25.8	13.87 ±1.06	4.70 ±0.67	9.17 ±0.63	7.66 ±0.29	6.43 ±0.27
Fed + 1.5 mg GH/72 hours	11	223 ±21.8	+12.4 ± 1.1	828 ±37.1	2.34 ±0.36	587 ±20.0	8.71 ±0.68	1.90 ±0.13	6.81 ±0.56	8.43 ±0.19	6.12 ±0.13
Fasted 24 hours + 1.5 mg GH/72 hours	16	179 ±13.7	−24.0 ± 1.7	695 ±83.9	3.04 ±0.41	358 ±50.0	10.68 ±0.97	2.80 ±0.25	7.87 ±0.77	9.58 ±0.74	6.81 ±0.22

[a] All GH injections were 0.25 mg ip twice daily for either 1 day or 3 days as indicated.

[b] Mean ± SE.

did not alter the nitrogenous blood components of the diabetic rats in any consistent manner. It should be noted that at no time did fasting increase cardiac glycogen in the diabetic rat.

Of interest is the last group in Table V which was injected with 1.5 mg of growth hormone over a 3-day fasting period. The data show extremely large standard errors for most variables measured. Close inspection of the individual data clearly revealed two distinct groups of animals, one which had cardiac glycogen levels exceeding 750 mg/100 gm tissue and a second group which had cardiac glycogen levels markedly below 500 mg/100 gm tissue. The high cardiac glycogen group had liver glycogen, plasma glucose, and total ketone levels which were almost double those of the low glycogen group (Table VI). Also, body weights and plasma amino nitrogen levels were greater in the high cardiac glycogen group. In essence, these two groups appeared to be very similar to the groups of depancreatized rats described by Scow *et al.* (21,24). They reported such differences in diabetic rats subjected to fasting and attributed the ketonemia to hypophyseal secretions. Also, in accord with Scow's data was the observation herein that the smaller rat lost more weight but appeared healthier than the heavier rats; these observations were associated with a lower ketonemia than that found in the heavier animals.

Discussion. The data presented in Tables II–IV and Fig. 1 clearly indicate a close parallel between cardiac glycogen levels and plasma total ketone bodies. A high positive correlation exists between these two parameters in fasted intact rats as well as in fasted and fed alloxan-diabetic rats. A much less reliable correlation was found relating cardiac glycogen with plasma glucose levels. These observations complement and extend those from Evans' laboratory who found that isolated rat hearts from starved and diabetic animals demonstrated reduced oxidation of palmitate-^{14}C concomitant with a markedly increased incorporation of the label into tissue lipid (4). From this and other work he concluded that a greater lipolysis of preformed endogenous tissue lipid occurs in the diabetic

TABLE VI. Reevaluation of 24-Hour Fasted Diabetic Rats Injected with 1.5 mg GH over 72 Hours: Grouped According to Cardiac Glygogen Levels.

Group	No. of obs.	Body wt. (gm)		Tissue glycogen		Plasma analyses (mg/100 ml of blood)					
		Initial	Change	Heart (mg/100 gm)	Liver (%)	Glucose	Total ketones	Aceto-acet.	β-Hydroxy.	Amino N_2	Protein (%)
Low cardiac glycogen group (less than 750 mg/100 gm)	7	148 ±13.4[a]	−27.1 ±1.67	349 ±47.2	1.16 ±0.31	235 ±22.7	7.38 ±0.85	2.06 ±0.27	5.32 ±0.90	7.90 ±0.13	6.42 ±0.26
High cardiac glycogen group (over 750 mg/100 gm)	9	204 ±12.8	−20.0 ±1.12	963 ±39.4	3.20 ±0.27	440 ±27.4	12.73 ±1.11	3.27 ±0.37	9.11 ±0.78	10.69 ±0.58	7.03 ±0.28
Combined groups[b]	16	179 ±13.7	−24.0 ±1.70	695 ±83.9	3.04 ±0.41	358 ±50.0	10.68 ±0.97	2.80 ±0.25	7.87 ±0.77	9.58 ±0.74	6.81 ±0.22

[a] Mean ± SE.

[b] Same group as in Table V; consult footnote for injection regimen.

rat heart. Starvation produces similar alterations in myocardial metabolism but to a lesser extent, observations which are in accord with those presented herein. Fasting cardiac glycogen levels in intact rats, regardless of the duration of the abstinence period, were elevated above normal levels but never approached the much higher levels observed in nonfasted diabetic rat hearts, regardless of severity of the diabetic state (Table III). Further evidence relative to these observations has been presented indicating that rat heart muscle preferentially utilizes fatty acids while suppressing glucose-^{14}C oxidation and glycogenesis (5). Thus, it would appear that oxidation of fatty acids from endogenous sources "regulates" utilization of carbohydrate substrates (4). Both fasting and diabetes markedly increase myocardial lipoprotein lipase which specifically hydrolyzes the triglyceride component of low density lipoproteins (27). The end result is increased fatty acid metabolism and ketone body formation in both starvation and diabetes resulting in a sparing of cardiac glycogen.

Studies in intact as well as hypophysectomized–depancreatized mammals indicate that cardiac glycogen levels are largely independent of insulin availability (25). However, the action of growth hormone on cardiac glycogen is better established (9,11) and is thought to make available ketone bodies for myocardial fuel, rather than as substrates for glycogen, thereby suppressing glycogenolysis. As reported herein, fasting of diabetic rats does not increase further the already elevated cardiac glycogen levels. This may be due to the previously reported diminished growth hormone levels in the diabetic rat (12,13) or may be due to a decreased responsiveness of myocardial tissue to the anabolic hormone since exogenous growth hormone was without glycogenic effect in either fed or fasted diabetic animals (Table V). An accord is found between data demonstrating progressive loss of hypophyseal growth hormone content in rats during a 96-hour fast (26) and the progressive rise in fasting cardiac glycogen over the same time period (9). Critical estimates of circulating growth hormone during this time are lacking, however. It is of interest to note that in contrast to the apparent independence of growth hormone from insulin on cardiac glycogen in the rat (9,11) there is very good evidence that insulin is required for the action of growth hormone in cardiac glycogen deposition in several strains of mice (28). Finally, it would appear that sparing of cardiac glycogen with concomitant increased fatty acid metabolism in the face of diminished levels of pituitary and circulating growth hormone in the diabetic rat suggests altered tissue enzyme activity independent of normal circulating somatotrophin levels.

Summary. The influence of growth hormone, plasma glucose, and plasma ketone bodies on cardiac glycogen deposition was studied in fasted intact rats and both nonfasted and fasted alloxan-diabetic rats. The results indicate a close positive correlation between plasma ketones (but not plasma glucose) and cardiac glycogen. Fasting does not increase diabetic myocardial glycogen levels and, unlike normal intact rats, growth hormone did not influence myocardial polysaccharide levels in diabetic rats indicating a possible tissue refractoriness to the hormone or the need for "permissive" amounts of insulin to be present.

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Autoregulation of Adipose Tissue Mass in the Mouse* (32714)

ROBERT A. LIEBELT, L. VISMARA, AND A. G. LIEBELT (Introduced by W. J. Schindler)
Department of Anatomy, Baylor University College of Medicine, Texas Medical Center, Houston, Texas 77025

Lipid deposition occurs at different rates in the inguinal and gonadal fat depots of NH and CBA mice during postnatal development, yet the lipid content of either fat depot represents a direct proportion of the total body lipid content during normal growth or the development of goldthioglucose-induced obesity (1,2). Surgical removal of the gonadal fat depots followed by a single injection of goldthioglucose results in a "compensatory hypertrophy" of the remaining fat depots during the development of obesity and an increased rate of converting dietary constituents into stored lipids (3). These data suggest that the anatomically dispersed fat depots are integrated into a functional total adipose tissue mass.

Syngeneic grafts of ovarian (4) and splenic (5) tissues atrophy in intact animals. However, ovarian and splenic grafts remain morphologically and functionally viable in ovariectomized or splenectomized hosts, respectively. Interpretations of these findings favor the existence of autoregulatory mechanisms determining tissue or organ mass (6). It was the purpose of the following studies to determine the fate of syngeneic adipose tissue grafts in intact mice and in mice in which a deficit in the total adipose tissue mass was created by surgical removal of specific fat depots.

Materials and Methods. Experiment A. A total of 84 (BALB/c Ki × CE/Ki) F_1 hybrid mice[1] of both sexes were used at approximately 90 days of age and were distributed into four comparable groups according to age, sex, and body weight. All animals were anesthetized with sodium pentobarbital and the peritoneal cavity was opened by a midline incision from the xiphoid process to the region of the external genitalia. Animals assigned to Group I had the paired epididymal or parametrial fat depots (GFO's) in males and females, respectively, lifted from the abdominal cavity without interfering with the blood supply and returned to the normal anatomical position. Group-II animals had one of the paired fat depots partially transected lateral to the main blood supply (tes-

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