toxifies bacterial endotoxin has been prepared from normal dog spleen. The detoxifying system is thermolabile, precipitable with ammonium sulfate, completely inactivated by trypsin and by tetraethyl pyrophosphate. These findings suggest that the active component is a protein with the properties of an esterase type of enzyme.

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Comparative Expiratory and Oxidative Effects of Glucagon and Epinephrine in Mice. (28491)

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Subcutaneous injection of glucagon has been reported to increase metabolic rate of rats, similarly to epinephrine(1). In fact, it has been proposed that in rats and dogs, glucagon may cause secretion of epinephrine from adrenal glands(2,3). Recent experiments in our laboratory have been concerned with the general metabolic effects of glucagon and epinephrine in mice. The present report suggests that, in contrast to epinephrine, glucagon did not increase metabolic rate of mice. However, glucagon appeared to increase oxidation rate of C^{14}-glycerol by nonfasted mice.

Materials and methods. Ad libitum fed male albino rats (Charles River) and mice (Upjohn colony-Rockland Farm ancestry or Carworth Farm CF_1) weighing 165-175 g and 25-28 g respectively were used. Certain mice (Upjohn colony) were fasted 15 hours (overnight) before use. Diabetes was induced in mice by intravenous (IV) injection of 70 mg/kg alloxan-monohydrate as a 1% aqueous solution(4). Typical fasting blood glucose values obtained from diabetic mice were about 300 mg% when placed on experiment 48 hours after alloxan injections. Blood for glucose analysis was obtained by cardiac puncture of mice under light ether anesthesia. Blood glucose was determined by an Auto-Analyzer* utilizing a method reported previously(5) but modified in that protein-free filtrates were obtained by dialysis. Liver glycogen was determined by an anthrone method (6). Fresh preparations of hormones were made daily and injected as indicated; control animals received appropriate vehicle alone. Glucagon† was suspended in 0.25% methyl cellulose aqueous vehicle and 0.2 ml of the appropriate concentration injected subcutaneously (SC) or intraperitoneally (IP) into rats or mice. Epinephrine‡ was given SC (0.37 mg/kg) in 0.1 ml 0.9% saline to rats or mice.

For expiratory experiments, a hormone or vehicle alone was injected 40 minutes before measurements of expired CO_2 were begun. In

* Technicon Instrument Corp., Chauncey, N. Y.
† Eli Lilly & Co., Lot 258-234B-167-1.
‡ Parke-Davis & Co., Adrenalin 1:1000.

TABLE I. Effect of Glucagon on Blood Glucose and Liver Glycogen of Intact Nonfasted Mice.

Group	Blood glucose (mg %) 1 hr	p	3 hr	Liver glycogen 2 hr	p
(10)* Controls	160 ± 13†		130 ± 18		
" Glucagon‡	225 ± 15	<.01	145 < 13		
(8) Controls				4.8 ± .5	
" Glucagon‡				.7 ± .1	<.01

* No. of Upjohn colony mice used. Blood glucose values for same mice at 1 hr and 3 hr for control and glucagon treated.

† Standard error of mean; (p) Probability that differences between observed means and controls are due to chance.

‡ SC glucagon, 3.8 mg/kg.

certain experiments, each mouse was also injected with a C^{14}-labeled compound immediately before measuring expired CO_2 and $C^{14}O_2$ as follows: 0.13 μc C^{14}-1,3-glycerol IP in 0.1 ml of 0.9% saline or 0.1 μc C^{14}-1-palmitic acid IV in 0.1 ml mouse serum. A group of 3 mice or 1 rat (control or treated of equal weights alternating throughout the day) was placed in a glass metabolic chamber for 1 hour and total exhaled CO_2 and $C^{14}O_2$ measured by a continuous gas analyzer§ utilizing an infrared analyzer and an ion chamber electrometer to monitor the respective gases. Expired CO_2 was considered indicative of metabolic rate (MR) and values were calculated in millimoles (mM) on the basis of standard conditions of temperature and pressure (STP) compared to known CO_2. Values for expired $C^{14}O_2$ were calculated as counts per minute (cpm) based on results from a gas flow counter used for standardization where 1 μc = 4.4×10^5 cpm.

Results and discussion. Exhaled CO_2 from rats and mice. Subcutaneous injection of 3.8 mg/kg glucagon increased exhaled CO_2 of rats 27% but did not increase exhaled CO_2 of Upjohn colony mice (Fig. 1). Lack of MR increase for mice was rather unexpected because of reported stimulatory effects of glucagon on MR of rats given similar doses mentioned earlier(1). Injecting glucagon IP at 3.8 mg/kg or increasing the SC dose to 7.6 mg/kg was also ineffective in altering MR of Upjohn colony mice. Exhaled CO_2 from Carworth Farm mice given SC glucagon was also unchanged (Fig. 1, footnote). With regard to a MR effect of epinephrine, Upjohn colony mice responded in a predictable fashion by exhaling CO_2 at an increased rate of 39% and an equivalent dose of epinephrine increased CO_2 output of rats by 35% (Fig. 1). The results shown here for rats are similar to those reported(1).

The reason for apparent inability of glucagon to raise MR of mice used is unknown. Sufficient hormone was absorbed from SC injection site to produce other well known metabolic effects, during a time sequence similar to that employed for expiratory measurements. Glucagon increased blood glucose from 160 to 225 mg% (40%) 1 hour after injection and decreased liver glycogen from 4.8 to 0.7% (85%) after 2 hours (Table I). It may be that higher doses of glucagon would have finally increased MR of mice,

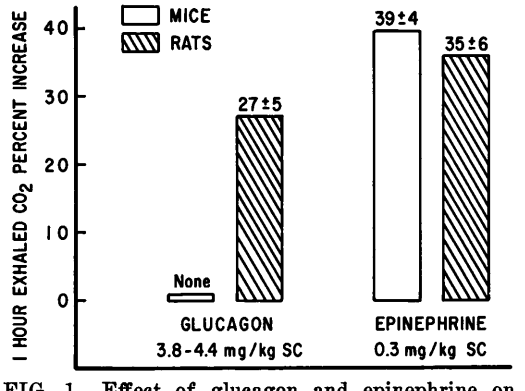

FIG. 1. Effect of glucagon and epinephrine on exhaled CO_2 of nonfasted mice and rats. Each value is an avg for 8-10 determinations ± standard error of mean. Glucagon did not alter exhaled CO_2 of Upjohn colony mice (3.8 or 7.6 mg/kg SC and 3.8 mg/kg IP) or Carworth Farm mice (3.8 mg/kg SC).

§ Model 2000, General Measurements, Inc., Garnerville, N. Y.

TABLE II. Oxidative Responses of Mice Having Hyperglycemia Induced by Various Agents.

Group	Blood glucose, % increase at 1 hr	12 min cumulative SA†			
		C^{14}-glycerol		C^{14}-palmitic acid	
		cpm/mM	p‡	cpm/mM	p‡
Controls		492 ± 96		1094 ±138	
Glucagon	40	948 ±124	<.05	544 ± 92	<.01
Glucose	35	536 ± 98	NS	704 ± 42	<.05
Epinephrine	109	340 ± 62	<.20	1402 ± 56	<.10
Diabetic	105*	718 ± 92	<.10	1616 ± 94	<.05

Nonfasted Upjohn colony mice, 10 determinations per glucose value (10 mice) or SA value (30 mice) above; blood glucose values 1 hr after injection of 3.8 mg/kg SC glucagon, 100 mg glucose/0.2 ml H_2O IP/mouse or 0.37 mg/kg SC epinephrine.

* Blood glucose increase 48 hr after IV alloxan.

† Cumulative specific activity in counts/min/millimole exhaled CO_2 ± standard error of mean. Cumulative indicates total $C^{14}O_2$ exhaled from injection time 0. Epinephrine increased exhaled CO_2 and decreased total C^{14}-glycerol oxidized.

‡ (p) Probability, (NS) not significant.

but it is unlikely since the SC dose of 7.6 mg/kg used here was 20 times that found effective in rats(1). It has been reported that for rats both cortisone and thyroid hormone were prerequisites for maximal increased MR effects produced by glucagon or epinephrine; hyperglycemia *per se* was ineffective(1,7). On the other hand, from MR data reported here showing intact mice to respond to epinephrine, but not to glucagon, a definition of more exact relationships existing between all of these hormones with regard to MR awaits further experimentation.

Oxidation of C^{14}-glycerol and C^{14}-palmitic acid in mice. Both glucagon and epinephrine appear to promote fatty acid oxidation in adipose tissue *in vitro* and in the intact animal(8). This general effect on lipid metabolism may be related to increased MR associated with hormone administration. However, even though glucagon did not increase MR of mice, it was of interest to determine if glucagon would alter oxidation of representative lipid components in mice. Results of a comparative study on effects of both glucagon and epinephrine on oxidation of C^{14}-glycerol and C^{14}-palmitic acid are shown in Table II. Specific activity of exhaled CO_2 from mice given glucagon and C^{14}-glycerol increased from 492 to 948 cpm/mM (92%), which was at a peak during the first 12 minutes after C^{14} injection. On the other hand, specific activity of CO_2 resulting from C^{14}-palmitic acid oxidation was lowered from 1094 to 544 cpm/mM (36%) by glucagon during the 12 minute interval. Glucose injection did not alter C^{14}-glycerol oxidation, although it produced hyperglycemia and decreased C^{14}-palmitic acid oxidation similar to glucagon (Table II). Glucagon influence on C^{14}-glycerol oxidation was, therefore, independent of hyperglycemia *per se* since glucose-induced hyperglycemia did not increase C^{14}-glycerol oxidation. Decreased oxidation of C^{14}-palmitic acid probably resulted from increased carbohydrate utilization, in response to hyperglycemia initiated by glucose(9) or glucagon treatment.

Epinephrine appeared to decrease specific activity of exhaled CO_2 from 492 to 340 cpm/mM (30%) ($p = <0.20$) after C^{14}-glycerol injection; when C^{14}-palmitic acid was given, specific activity was apparently increased by epinephrine from 1094 to 1402 cpm/mM (28%) ($p = <0.10$) (Table II). These effects were opposite to those obtained after glucagon administration. If it is assumed that, as in rats(10) mice used here rapidly converted significant amounts of C^{14}-glycerol to blood glucose, effects of epinephrine shown above are in agreement with a generally accepted view that epinephrine decreases oxidation of glucose to CO_2 and increases oxidation of fatty acids(11). Diabetic mice oxidized both labeled compounds at an increased rate compared to nondiabetic controls (Table II). These results are in accord with numerous observations in-

TABLE III. Glucagon and Oxidation of C^{14}-Glycerol by Mice in Certain Metabolic States.

Group	Cumulative specific activity† 12 min cpm/mM	p‡	1 hr cpm/mM
Nonfasted			
(10)*Controls	492 ± 96		802 ± 32
" Glucagon	948 ± 124	0.05	930 ± 44
Fasted			
(5) Controls	864 ± 70		2640 ± 340
" Glucagon	1006 ± 114	NS	2496 ± 280
NF adrenal-X			
(4) Controls	642 ± 40		1060 ± 152
" Glucagon	1012 ± 58	0.01	1128 ± 150
F adrenal-X			
(4) Controls	840 ± 60		1362 ± 168
" Glucagon	888 ± 106	NS	1426 ± 120
NF diabetic			
(6) Controls	718 ± 36		1242 ± 94
" Glucagon	1176 ± 150	0.05	1336 ± 176

* No. of groups of Upjohn colony mice (3 mice/group). Nonfasted (NF), fasted (F), and adrenalectomized (adrenal-X); 3.8 mg/kg SC glucagon.

† Avg specific activity of exhaled CO_2 from injection time 0 to 12 min or 1 hr. During the 12 min or 1 hr interval, total CO_2 exhaled was unchanged by glucagon for all groups.

‡ Probability that differences between observed mean and control for each group is due to chance.

dicating increased triglyceride oxidation in the diabetic state due to lack of available insulin(9).

Glucagon stimulation of C^{14}-glycerol oxidation appeared to be related to initial C^{14}-glycerol utilization rather than to presence of adrenal hormones or insulin (Table III). Thus at lower rates of C^{14}-glycerol oxidation, as in nonfasted intact or adrenalectomized mice (492 and 642 cpm/mM) glucagon increased C^{14}-glycerol oxidation (948 and 1012 cpm/mM). However, when similar mice were fasted, baseline C^{14}-glycerol oxidation increased and glucagon had no significant effect. Glucagon increased C^{14}-glycerol oxidation by nonfasted adrenalectomized and diabetic mice only 57 and 63% respectively. Initial oxidation rates of these mice were somewhat greater than for nonfasted intact healthy controls and responses to glucagon were not as marked. Finally, the stimulatory effect of glucagon on C^{14}-glycerol oxidation in all responsive mice was brief (Table III). This is shown for example by results from nonfasted intact mice, where an increase in SA of exhaled CO_2 at 12 minutes (948 *vs* 492 cpm/mM) was reduced to little or no significance by 1 hour (cumulative SA 930 *vs* 802 cpm/mM). No increase in exhaled CO_2 was observed from glucagon treated mice during the first 12 minute period (Table III, footnote).

In conclusion it is suggested that in mice injected glucagon is associated with glycerol oxidation. This proposed action is not dependent on hyperglycemia *per se,* adrenal hormones or insulin, but is dependent on initial glycerol oxidation rate.

Summary. In confirmation of other studies, SC injection of glucagon increased exhaled CO_2 (apparent metabolic rate) of rats. Contrary to expectation, SC and IP injection of glucagon did not increase exhaled CO_2 of mice. Glucagon did, however, produce expected loss of liver glycogen and hyperglycemia in mice. Subcutaneous injection of epinephrine increased exhaled CO_2 of both rats and mice.

Glucagon appeared to increase oxidation rate of C^{14}-glycerol and decrease oxidation of C^{14}-palmitic acid; opposite effects were obtained with epinephrine. The "glucagon effect" on C^{14}-glycerol oxidation was brief and dependent on initial utilization of C^{14}-glycerol, as fasting mice with greater oxidation rates did not respond. This oxidative stimulation was not dependent on hyperglycemia *per se,* adrenal hormones or insulin.

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