Effect of Glucagon and of Insulin on Serum Free Fatty Acids in Normal and Depancreatized Dogs* (33487)

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The concentration of free fatty acids (FFA) in the serum is influenced by a variety of stimuli and hormones, including glucagon and the catecholamines. The primary effect of these hormones appears to be a cyclic AMP-mediated activation of tissue lipase resulting in the net mobilization of FFA from adipose tissue and liver (1–6). In the case of the catecholamines this mobilization consistently increases serum FFA concentration (7,8) while in the case of glucagon, the results have been contradictory. For example, glucagon decreased the concentration of FFA in the liquid circulating through a perfused rat liver (9), while the intraportal administration of glucagon in unanesthetized dogs decreased serum FFA concentration (10), but increased it in a similar experiment in which anesthetized dogs were used (11). Indeed, in intact animals, parenteral glucagon may cause an increase (12, 13), a decrease (15–17) or a diphasic change (18) in serum FFA level. The interpretation of these findings is difficult, as they are the result of multiple and, sometimes, opposing forces, such as the stimulation of lipolysis by glucagon itself and by the catecholamines which it releases and the inhibition of lipolysis by glucagon-induced hyperglycemia and insulin release (5, 11, 19, 20). Other factors may be the length of the preexperimental fast (7, 18, 21), the presence or absence of anesthesia (10, 11), the degree of sensory stimulation (22) and of muscular activity (23), the animal species (13, 14), the presence or absence of the pancreas (16) or of diabetes (15, 24) and, possibly, the amount of insulin contained in the glucagon preparation and the timing of the samples. These apparent contradictions and unanswered questions provided the stimulus to undertake the present study in normal and depancreatized dogs, trained and unanesthetized.

Materials and Methods. Thirteen mongrel dogs of both sexes, weighing 6–25 kg, were housed in individual metabolic cages and fed a commercial diet twice a day. Pancreatectomy was performed under pentobarbital anesthesia and, unless stated otherwise, the dogs were used only after they had recovered from the operation and had resumed their normal eating habits. Severe glycosuria and ketonuria, 1–3 days after surgery, were considered tentative evidence of total pancreatectomy until confirmation at autopsy. Urinary glucose and ketone bodies were determined semiquantitatively using Clinistix and Acetest Tablets.[2] When positive, urinary glucose was measured quantitatively with the Benedict reagent. All dogs were trained to lie quietly for approximately 4 hr. No visitors were allowed during the experiment. An indwelling catheter, placed in the external jugular vein approximately 0.5 hr before the start of the experiment, served for hormone injection and the removal of blood samples. After each sample of blood was collected, an equal volume of saline (usually 10 ml) was injected. Glucose was determined according to Nelson (25), and FFA according to Dole and Meinertz (26). The immuno-reactive insulin (IRI) content of serum and of the glucagon preparations was determined according to Hales and Randle (27). The statistical significance of the results was calculated according to Snedecor (28). Forty-one experiments were performed in 13 animals: some dogs were studied before and after total pancreatectomy but before receiving insulin treatment, other dogs were maintained on lente and/or regular insulin and were studied

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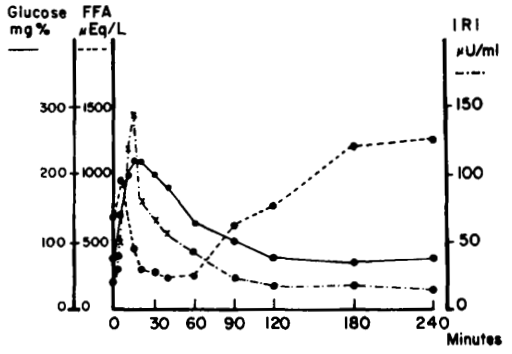

FIG. 1. Effect of commercial glucagon in 6 normal dogs.

40 hr after the last insulin injection. In 9 of these experiments, the animals received saline instead of either glucagon or insulin. Urinary glucose and ketone bodies were determined daily in 24-hr urine samples, as a guide to insulin dosage. All animals were fasted 24 hr before the experiment, except one dog which was fasted 96 hr. The following hormone preparations were used: "glucagon-free" crystalline insulin (Lot No. PJ-4609),[3] commercial glucagon (Lilly; insulin content 5 mU/mg); cysteine-treated glucagon (Lilly,[4] lot No. 258-234B-177; insulin content 0.25 mU/mg) and two preparations of cysteine-treated glucagon (Novo;[5] insulin content 0.25 and 0.5 mU/mg). The hormones were injected in a volume of 0.1 ml/kg.

Results. 1. *Commercial glucagon.* Figure 1 shows that the injection of commercial glucagon (0.1 mg/kg, i.v.) into normal dogs resulted in a rapid increase of serum FFA reaching a maximum in about 5 min. From this elevated level, FFA declined to a low value after 40–60 min then rose again reaching a second maximum 3–4 hr after the injection. As expected, glucagon caused a marked and rapid hyperglycemia, with a maximum in 15 min. At the end of 2 hr the glucose level had returned to baseline. We also noted a rapid increase of serum IRI which, starting at a value of 21 ± 1.0 µU/ml, rose to a peak of about 146 ± 8.5 µU/ml (611% ± 65 SE; $p < 0.01$) in 15 min and returned to the starting level in 1 hr. It will be noted that the decrease in serum FFA concentration coincided with the rapid rise in serum glucose and IRI levels and that the subsequent rise in serum FFA occurred at the time when both serum glucose and IRI were falling.

Figure 2 shows the results obtained in depancreatized dogs treated with insulin until 16 hr before the experiment. In these animals the mean fasting serum fatty acid value was higher than in normal dogs. Following the injection of commercial glucagon, the serum FFA level rose to a maximum in 10 min, decreased at the time of maximum hyperglycemia and finally returned to baseline values toward the end of the experiment. Figure 3 shows the responses to commercial glucagon obtained in depancreatized dogs 40 hr after the withdrawal of insulin. These animals had elevated fasting levels of serum glucose and FFA; nevertheless, both were increased further by glucagon. Following this rise, the serum FFA level decreased to a minimum in about 40 min at the time of, or shortly after, the hyperglycemic peak. At the end of the experiment the concentration of FFA and of glucose was returning toward, but had not reached the baseline. Figure 4 shows the results obtained in ketotic depancreatized dogs which had never been treated with insulin. In these animals, used 3 or 4 days after surgery, the average fasting serum FFA and

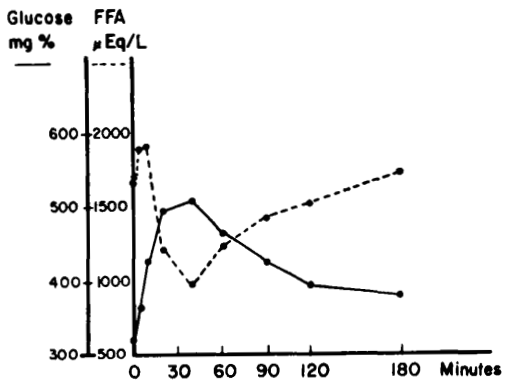

FIG. 2. Effect of commercial glucagon in 5 insulin controlled depancreatized dogs.

[3] Gift of Dr. Mary Root, Lilly Research Laboratory, Indianapolis.

[4] Gift of Dr. William Bromer, Lilly Research Laboratory, Indianapolis.

[5] Gift of Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen.

TABLE I. Effect of Commercial Glucagon on Serum FFA Concentration.

Condition of dog	Initial level	5–10 min	40–60 min	3–4 hr
	(μeq/liter; av ± SE)			
Normal (6)	705 ± 15	952 ± 39[b]	242 ± 20[b]	1263 ± 76[b]
Av diff. (% ± SE)		34 ± 3.5	64.5 ± 3.1	95 ± 7
Depancreatized controlled with insulin (5)	1600 ± 236	1917 ± 286[b]	967 ± 155[b]	1732 ± 126
Av diff. (% ± SE)		19.2 ± 0.8	43 ± 3.3	17.7 ± 7.2
Depancreatized off insulin 40 hr (10)	3222 ± 152	3446 ± 163[b]	2641 ± 149[b]	2937 ± 68
Av diff. (% ± SE)		8 ± 1.3	18.1 ± 1.8	7.1 ± 4.5
Depancreatized never treated with insulin (4)	2603 ± 160	2779 ± 157[a]	2315 ± 139[b]	2256 ± 52
Av diff. (% ± SE)		7.2 ± 0.6	10.9 ± 0.5	11.9 ± 4.6

[a] <0.01.
[b] <0.001.

glucose concentrations were elevated. A triphasic FFA response to commercial glucagon was noted again, although the changes were not as marked as those obtained in the normal and in the insulin-treated depancreatized animals. The average serum glucose concentration did not rise until the end of the experiment. The circulating IRI (9–12 μU/ml) was significantly lower than that found in normal dogs, but had not disappeared entirely. No increase occurred following glucagon injections. The maximal FFA and glucose responses to glucagon are summarized in Tables I and II. Note that the serum FFA level rose in all groups of animals, even though the initial value varied greatly from group to group.

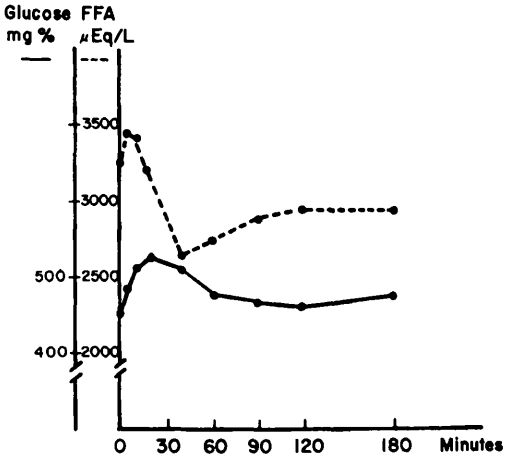

FIG. 3. Effect of commercial glucagon in 10 depancreatized dogs, 40 hr after the last insulin injection.

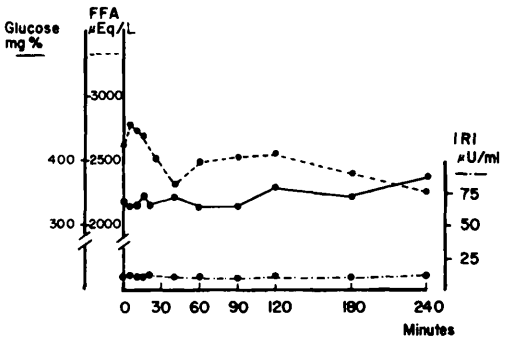

FIG. 4. Effect of commercial glucagon in 4 depancreatized dogs which had never received insulin.

The lot of commercial glucagon used in these experiments contained 5 mU of IRI/mg. This meant that the dogs had received 0.5 mU of insulin/kg of body weight. Two experiments were done to determine the effect of this dose of insulin in depancreatized dogs which had received no insulin treatment for 40 hr. Although the decrease in serum glucose concentration was minimal, the serum FFA concentration fell markedly

TABLE II. Effect of Commercial Glucagon on Serum Glucose Concentration.

Condition of dog	(mg/100 ml; av ± SE) Initial level	5–10 min	40–60 min	3–4 hr
Normal (6)	74 ± 4	200 ± 9[b]	179 ± 10[b]	75 ± 3
Av diff. (% ± SE)		192.0 ± 35.3	183.1 ± 29.0	19.0 ± 7.9
Depancreatized controlled with insulin (5)	320 ± 41	425 ± 45[b]	508 ± 36[a]	380 ± 16
Av diff. (% ± SE)		42.5 ± 4.2	87.4 ± 23.2	46.4 ± 22.0
Depancreatized off insulin 40 hr (10)	450 ± 12	509 ± 14[c]	511 ± 23[c]	474 ± 62
Av diff. (% ± SE)		13.5 ± 1.9	13.7 ± 2.8	5.6 ± 10.6
Depancreatized never treated with insulin (4)	334 ± 4	329 ± 4[a]	342 ± 3	374 ± 4[b]
Av diff. (% ± SE)		1.6 ± 0.5	2.3 ± 1.0	12.0 ± 1.4

[a] <0.05.
[b] <0.01.
[c] <0.001.

within 15 min and remained low for the remainder of the experiment. Only when the dose of insulin was reduced to 0.05 mU/kg did the serum FFA and glucose concentration fail to decrease (Table III). The possible role of insulin present as an impurity was investigated further using two preparations of cysteine-treated glucagon. In these experiments the total quantity of insulin administered to the animal was 25 μU/kg or half of that dose which had no demonstrable effect on the concentration of serum FFA and glucose: the results were similar to those obtained with commercial glucagon (Figs. 5, 6, 7). The injection of saline (0.1 ml/kg) did not alter the concentration of serum glucose and FFA significantly in two normal, in three insulin-controlled and in four noncontrolled diabetic dogs.

Discussion. The results of the experiments described in this paper indicate that, in the unanesthetized dog, the response of serum FFA concentration to glucagon was characterized by an initial rise, a fall and a second rise. It may be well to analyze these three phases of the response separately. The initial

TABLE III. Effect of Insulin or Cysteine-Treated Glucagon on Serum Glucose and FFA Concentration.

	Insulin dose (mU/kg)	Glucose (mg/100 ml) Initial level	5–10 min	% Δ	40–60 min	% Δ	180 min	% Δ
Diabetic off insulin (1)	0.5	321	345	7.5	331	3.1	356	10.9
Diabetic off insulin (1)	0.5	378	376	0.5	341	9.8	355	6.1
Diabetic on insulin (1)	0.05	27	27	0.0	24	11.1	—	—
Diabetic on insulin (1)	0.05	152	169	11.2	223	46.7	—	—
		FFA (μeq/liter)						
Diabetic off insulin (1)	0.5	2593	2146	17.2	2045	21.1	2016	22.3
Diabetic off insulin (1)	0.5	3523	2932	16.8	2793	20.8	2585	26.6
Diabetic on insulin (1)	0.05	684	745	8.9	1025	49.9	—	—
Diabetic on insulin (1)	0.05	1552	1529	1.5	1796	15.7	—	—

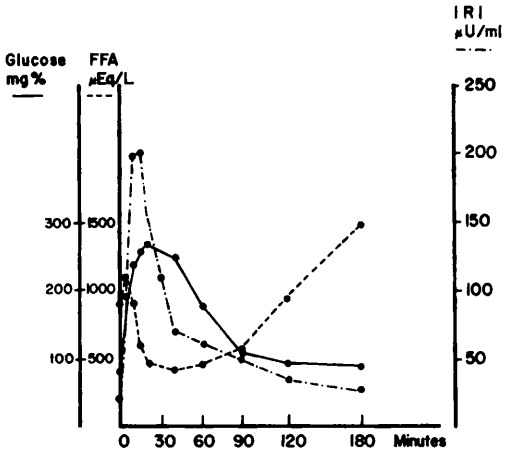

FIG. 5. Effect of cysteine-treated glucagon in 1 normal dog.

rise coincided with a rapid increase in the level of serum glucose and IRI and lasted 5–10 min. Its intensity and duration could not be modified significantly by pancreatectomy and did not depend upon the severity of the diabetic state or the initial serum FFA concentration. We believe that this rise may have been the result of a rapid stimulation of lipolysis by glucagon, which could not be prevented by the concurrent rise of glucose and IRI levels. It should be remembered that the action of glucagon *in vivo* is very rapid and that insulin injected intravenously can reduce, but not suppress, the hyperglycemic

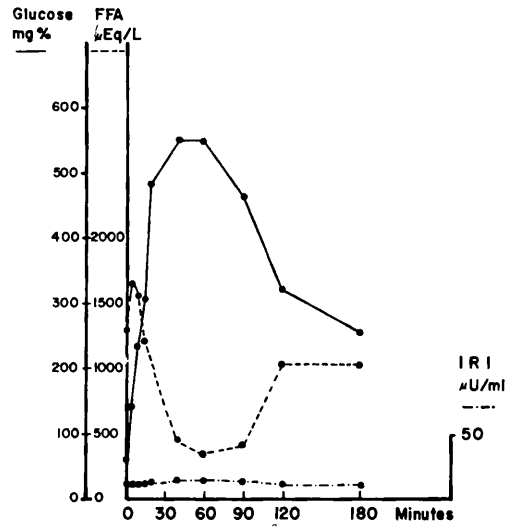

FIG. 6. Effect of cysteine-treated glucagon in 1 insulin controlled depancreatized dog.

effects of the small amounts of glucagon which most insulin preparations contain (29). It is possible that epinephrine and norepinephrine which, at least in man and in the rat, are released within a few minutes of an intravenous injection of glucagon (30), may have contributed to the initial rise in serum FFA. However, this explanation is not entirely satisfactory because the catecholamines which inhibit insulin secretion (10, 31–33), should have suppressed the serum IRI response. The fact that other investigators (15–17) did not observe the initial rapid rise in serum FFA may be explained by their failure to obtain samples during the

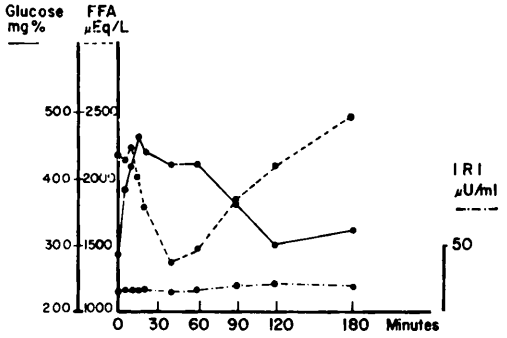

FIG. 7. Effect of cysteine-treated glucagon in the same dog as in Figure 6, 40 hr after the last insulin injection.

first 5 or 10 min of the experiment. We believe that the secondary fall of FFA noted in our experiments can be ascribed to glucagon-induced hyperglycemia and insulin release or to insulin contaminating the glucagon preparations. The relative importance of these factors varied with the experimental conditions: when commercial glucagon was used, the animals received a dose of exogenous insulin which, although insufficient to modify the concentration of serum glucose, was demonstrably effective in reducing serum FFA. In our experiments, this dose was 0.5 mU/kg, while the amount of insulin contaminating the doses of glucagon used by Sokal and collaborators (16) may be calculated to have been 0.1–2.5 mU/kg. In addition, normal dogs were under the influence of endogenous insulin released by glucagon, by hyperglycemia and possibly, by the initial rise in serum FFA level (34, 35). However,

insulin could not have been a factor in depancreatized dogs receiving cysteine-treated glucagon. In these animals, hyperglycemia appears to be a plausible explanation for the reduction in serum FFA, especially since this reduction seems to have been roughly proportional to the rise in glucose. Indeed, a fall in serum FFA concentration coincident with hyperglycemia is a well-known phenomenon (3, 7, 8, 19, 24, 36). The final increase in serum FFA, noted in normal dogs, is more difficult to explain: it may have been simply the manifestation of a continuing lipolytic stimulus which had been temporarily halted or reversed by the sharp increase in serum glucose and IRI. The failure of depancreatized dogs to demonstrate this final rise in FFA could have been due either to the continuing counter effect of their high serum glucose or, perhaps more likely, to the fact that, in these animals, the basal rate of lipolysis was already high and could no longer be effectively increased.

Summary and Conclusions. Intravenous injections of glucagon were followed by a triphasic response in serum FFA concentration: an immediate rise, probably reflecting the lipolytic effect of the hormone, a secondary depression probably caused by hyperglycemia and by exogenous and/or endogenous insulin and a final rise which may have been the result of continued glucagon-induced lipolysis. It may be concluded that although glucagon depresses serum FFA concentration *in vivo* by stimulating insulin and glucose release, under suitable circumstances, its lipolytic effect can be demonstrated by a rise in serum FFA. A dose of insulin, as small as 0.5 mU/kg, which caused little change in serum glucose, was found to be effective in reducing serum FFA concentration. This amount of insulin may be present in commonly used doses of cystalline glucagon and may contribute significantly to their biologic effects. The prompt and potent insulogenic action of glucagon *in vivo* was confirmed. Three to 4 days after total pancreatectomy, serum IRI had decreased but had not disappeared. In these animals, the IRI response to glucagon could not be demonstrated.

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Plasma 17-Hydroxycorticosteroid Response to ACTH in *M. mulatta*: Dose, Age, Weight, and Sex* (33488)

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The rhesus monkey has been used frequently in studies of the adrenocortical response to various experimental manipulations; however, relatively few data are available concerning the monkey's normal plasma 17-hydroxycorticosteroid (17-OHCS) response to ACTH. Harwood and Mason (1) reported the response of five monkeys injected i.v. with 4 and 16 mg/kg body weight of an ACTH preparation of unspecified activity, and Migeon *et al.* (2), presented data for three monkeys injected i.v. with 0.4 and 4 U of ACTH/kg of body weight. One purpose of the present report is to present data on the 17-OHCS response to ACTH for an extensive series of monkeys. For this purpose, intramuscular depots of ACTH gel were used, both for the convenience of injection and for the longer maintenance of high ACTH concentrations.

As factors of potential importance in the adrenocortical response to ACTH, data were analyzed for changes with respect to age, sex, weight, and rearing conditions. Age was clearly of importance, since previous reports from this laboratory had indicated a plasma 17-OHCS response to ACTH in infant monkeys larger than that in adults (3, 4). It therefore seemed of interest to determine the age range over which this response declined to adult levels, and whether this response changed further in old adults. The possibility of a sex effect in terms of higher responses to ACTH by female monkeys had been suggested by a few observations in this laboratory (unpublished), while weight as an inversely related correlate of adrenocortical response had been suggested from data obtained on the rat (5). Finally, adrenocortical studies of rats subjected to early handling (6) have suggested that early experience is an important factor in adrenocortical function, thus raising the possibility that monkeys reared from birth in laboratory cages might differ in adrenocortical responsivity from monkeys reared in the wild.

Procedure. One hundred and two rhesus monkeys, *Macaca mulatta*, ranging in age from 2 days to 15 years were used. To determine ACTH dosages which would elicit maximal plasma 17-OHCS rises, four monkeys, two of each sex at 4–5 years of age, were tested (in 1961) for their response to 4 and 8 units/kg of body weight of Acthar gel (Armour, 40 units/ml) injected intramuscularly. Another four adults, two of each sex at 7–10 years of age, were tested (in 1966) for their response to 2, 4, 8, and 16 USP units/kg of body weight of ACTH gel (Organon, 80 USP units/ml) injected intramuscularly. Both of these groups were restrained in primate

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