assay. In the 2 assay procedures, the degree of substrate saturation levels can be assumed to be similar except for the specific activity of the radio-tyrosine used. The probable reasons for the previously reported lower tyrosinase activities are: (a) incomplete extraction of enzyme, (b) loss of enzymatic activity by use of low speed centrifugation to obtain a clear homogenate, and (c) incomplete collection of the reaction product (melanin).

Summary. The tyrosinase activity in 4 varieties of goldfish (*Carassius auratus* L.), white, xanthic, grey and black moor, was determined. Tyrosinase activity occurred in both the soluble and particulate fractions of skin homogenates. Total tyrosinase activity was increased in both fractions with increasing melanin pigmentation. The activity increase, however, occurred, for the most part, in the particulate fraction. White and xanthic goldfish were identical in tyrosinase content and distribution. Tyrosinase activity in black moor skin is: skin to fin ratio, 4:1; dorsal skin to ventral skin ratio, 4:1; right side to left side ratio, 1:1. Further, smaller fish have a higher skin tyrosinase level than larger fish.

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Effects of Glucagon and Epinephrine in Fasted Dogs.* (30815)

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Glucagon and epinephrine have a major common effect *in vivo* and *in vitro*: liver glycogenolysis as evidenced by hyperglycemia. Another common effect of both hormones has been noted *in vitro*: lypolysis as measured by FFA release(1,2). This action of epinephrine has received innumerable confirmations with *in vivo* preparations(3,4), but a lipolytic action of glycagon in similar conditions has not been observed(5,6).

In vivo experiments may introduce several variables: length of fasting, diminishing the effectiveness of insulin(7); consciousness or type and duration of anesthesia, affecting the level of circulating cathecholamines(8). Since catecholamines are dominant factors controlling lipolysis and insulin is essential for lipogenesis(9), these variables may influence the basal state of the animal and need to be evaluated.

The purpose of this paper is to assess the effect of glucagon on plasma FFA *in vivo* and to compare it with that of epinephrine, taking into consideration the variables just mentioned.

Methods. Healthy mongrel dogs of both sexes, weighing 9-15 kg, were kept in metabolic cages on a regular diet for at least 2 weeks prior to the first experiment and thereafter. An interval of 2 weeks was allowed between experiments performed on the same dog. Experiments were carried out in anesthetized (pentobarbital sodium I.V. 35 mg/kg) and in unanesthetized animals. Two periods of fasting (24-hr and 48-hr) were stud-

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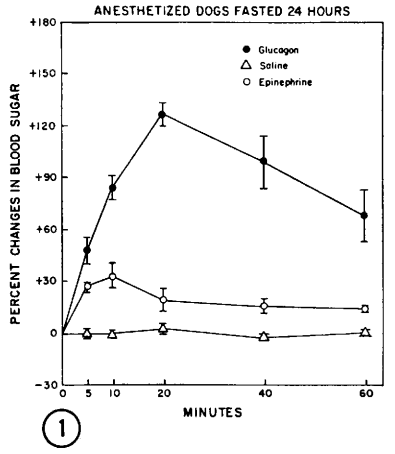

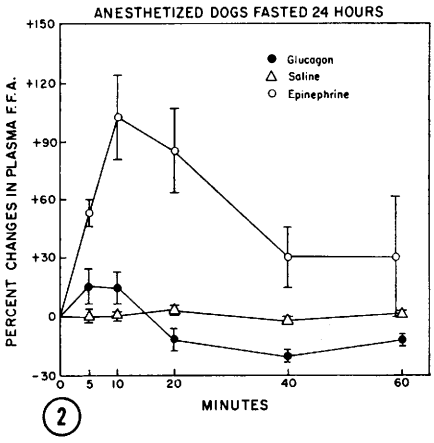

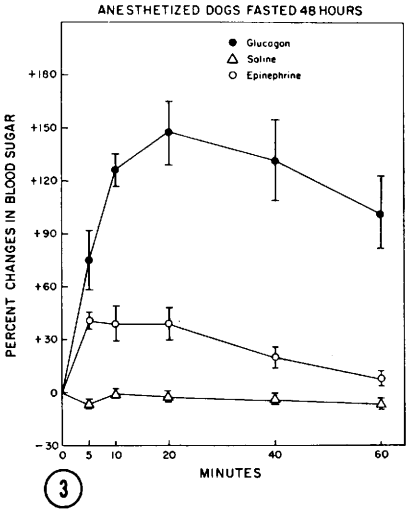

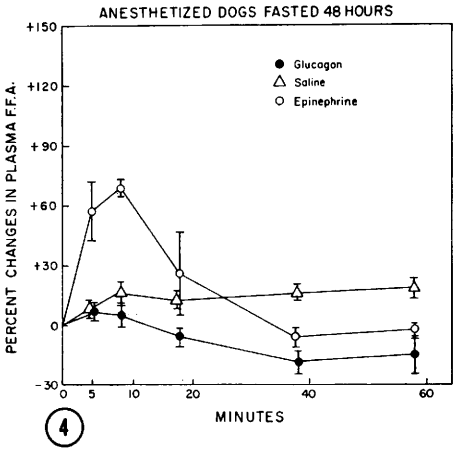

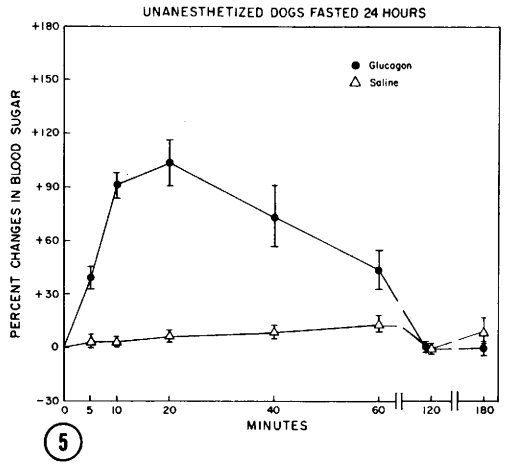

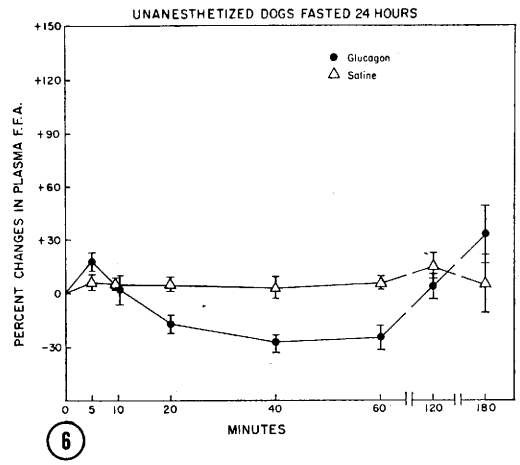

FIG. 1. Changes in Blood Sugar following injections of Glucagon (7 dogs), Epinephrine (5 dogs) and Saline (4 dogs).

FIG. 2. Changes in Plasma FFA following injections as in Fig. 1.

FIG. 3. Changes in Blood Sugar following injections of Glucagon (6 dogs), Epinephrine (5 dogs) and Saline (8 dogs).
FIG. 4. Changes in Plasma FFA following injections as in Fig. 3.
FIG. 5. Changes in Blood Sugar following injections of Glucagon (8 dogs) and Saline (5 dogs).
FIG. 6. Changes in Plasma FFA following injections as in Fig. 5.

ied in anesthetized dogs. Animals to be used in a conscious state were limited only to a fasting period of 24 hours. The hours of fasting were counted from the end of the last meal. Water was supplied *ad libitum* at all times. Daily urinalysis for ketone bodies was carried out on all animals during the experimental periods.

Crystalline glucagon‡ dissolved in glycine buffer (.5 mg per animal) epinephrine (10 μg/kg) or physiologic saline solution were injected into a peripheral vein. When unanesthetized animals were used, epinephrine injections were omitted to spare the conscious animals the systemic effects of the medullary hormone.

Blood samples were obtained from another peripheral vein, stored immediately in heparinized tubes placed in an ice bath. Aliquots for blood sugar determinations(10) were removed and the rest of the blood was centrifuged at 4°C. Plasma FFA determinations(3) were carried out promptly after centrifugation. Plasma total cholesterol was also measured(11). The period of observation was limited to one hour after injection of the test substance in view of the duration of primary effect of a biologically active peptide on lipolysis(12).

Results. Results are given in the Table and Figures. The Table presents average pre-injection values of blood sugar, plasma total cholesterol and FFA. Samples for these were taken 60 minutes, 30 minutes, and immediately before injections of the test substance. It can be noted that: (a) in comparing anesthetized animals fasting for 24 hours with animals fasting for 48 hours, there is a significant decrease ($P<0.01$) in blood glucose and a significant increase ($P<0.01$) in plasma FFA concentration. (b) Plasma cholesterol does not change significantly in any of the groups considered.

The curves represent changes from average pre-injection values in blood sugar and plasma FFA concentrations occurring after administration of the test substance.

The results show that, with the doses used, glucagon-induced hyperglycemia (Fig. 1) is far greater ($P<0.001$) than that caused by epinephrine ($P<0.01$). Hyperglycemic responses to both hormones are enhanced by a longer period of fasting (Fig. 3).

In animals fasted for 24 hours, epinephrine causes a quick and significant increase in FFA concentration (Fig. 2) reaching a maximum ten minutes after the injection ($P<.001$) with a gradual decline thereafter, not returning to pre-injection levels during the period of observation. In animals fasted for 48 hours, epinephrine causes a less marked increase in FFA (Fig. 4) concentration ($P<0.01$), which returns to control values 20 minutes after the injection. Glucagon injection is followed by a slow depression in concentration of plasma FFA, reaching significantly lower values ($P<0.01$) 40 minutes after the injection in either of the 2 groups considered (Fig. 2 and 4).

Results in unanesthetized animals are

TABLE I. Mean Pre-Injection Values (± S.E.).

	No. of dogs	Blood glucose (mg %)	Plasma total cholesterol (mg %)	Plasma FFA (μEq/l)
Unanesthetized fasted 24 hrs	13	76 ± 2	131 ± 7	512 ± 31
Anesthetized fasted 24 hrs	16	78 ± 1	146 ± 9	474 ± 31
Anesthetized fasted 48 hrs	19	71 ± 4	126 ± 8	564 ± 34

‡ Gift of Dr. Mary Root, Lilly Research Laboratories, Indianapolis, Ind.

given in Fig. 5 and 6. It is readily apparent from these curves that glucagon causes hyperglycemia not unlike that obtained in anesthetized dogs and is followed by a depression ($P<0.01$) in plasma FFA concentration similar to that observed in anesthetized animals.

No significant changes in plasma cholesterol were observed in any group of animals after administration of glucagon, epinephrine or saline. Ketonuria was never observed in any animal.

Discussion. These studies confirm previous observations that, in prolonged fast, blood sugar is depressed while FFA concentration is increased(13). The increase in FFA following administration of epinephrine observed in these experiments, after 24 hours of fasting, parallels that reported in the literature(3). The relatively smaller FFA response to the medullary hormone in the 48-hour fasting animals can be explained on the basis of continued lipolysis, thus reducing the fat stores available for additional breakdown after epinephrine injection.

These studies indicate that glucagon *in vivo* does not increase the level of FFA but produces a significant decrease in FFA in all experimental groups considered. These findings are in direct contrast with the reported action of glucagon *in vitro*. An explanation for this discrepancy may be that the direct action of glucagon *in vivo* is limited to only a few minutes(14), while the hormone remains in contact with the test tissue (fat pad) presumably during the duration of the experiment *in vitro*. The effect *in vivo* is restricted to the target organ, the liver, without any effective quantity of glucagon reaching other tissues(14), whereas *in vitro,* cells not physiologically exposed to this hormone are subjected to its action. The lowering of FFA after glucagon *in vivo* may therefore be attributed to the release of insulin, secondary to hyperglycemia, rather than to a direct effect of glucagon on adipose tissue.

We were unable to confirm the lowering of plasma cholesterol after injection of glucagon, observed by other authors(15).

Summary. The effect of glucagon on plasma FFA was studied in anesthetized dogs, fasting 24 or 48 hours and in conscious dogs fasting only 24 hours. Glucagon was found to depress FFA in all of these groups. In comparing this action with the opposite one seen after epinephrine injection, it is suggested that the effect of glucagon on plasma FFA is mediated by a secondary release of insulin.

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