Role of Lipolytic and Glucocorticoid Hormones in the Development of Diabetic Ketosis

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SUMMARY

Pancreatectomized rats hypophysectomized for one week or longer developed ketosis when given dexamethasone and growth hormone in amounts that were relatively inactive when administered alone. Growth hormone could be replaced by other lipolytic hormones, such as adrenocorticotrophic hormone (ACTH) and thyroxine.

When fasted hypophysectomized rats were made acutely diabetic by injection of mannoheptulose, the plasma concentrations of glucose, ketone bodies, free fatty acids (FFA) and glycerol were increased; injection of growth hormone and dexamethasone further increased the concentrations of these plasma constituents. Injection of these hormones increased lipolysis and decreased glucose metabolism, in vitro, by adipose tissue of mannoheptulose-treated hypophysectomized rats. Uptake of FFA and ketogenesis by perfused livers of fasted hypophysectomized rats were directly related to the concentration of FFA in the perfusing fluid. Growth hormone and glucocorticoid did not have an effect on FFA uptake and ketogenesis in the liver.

It is concluded that lipolytic and glucocorticoid hormones induce diabetic ketosis through their actions on adipose tissue. Lipolytic hormones stimulate lipolysis in adipose tissue while glucocorticoid limits, in the absence of insulin, re-esterification of FFA by suppressing the metabolism of glucose. The resultant increased release of FFA to the blood augments, in turn, the concentration of FFA in plasma, the uptake of FFA by the liver, and the formation of ketone bodies in the liver. DIABETES *21*: 946-54, September 1972.

Development of ketosis in diabetic rats can be prevented by administration of insulin or by removal of either the pituitary or adrenal glands.[1-3] The hormones needed for the development of ketosis in hypophysectomized diabetic animals vary with the species and with the duration of hypophyseal deficiency.[2-4] Injection of glucocorticoid causes ketosis in the hypophysectomized-pancreatectomized rat if given immediately after hypophysectomy whereas it is ineffective several days later unless given with growth hormone; growth hormone given alone is not ketogenic in the hypophysectomized-pancreatectomized rat.[2,5] Adrenocorticotrophic hormone (ACTH) is ketogenic in hypophysectomized but not in adrenalectomized diabetic rats, suggesting that this hormone causes ketosis, at least in part, by stimulating glucocorticoid secretion.[2]

In order to study the interrelationship between growth hormone and glucocorticoid in the development of diabetic ketosis, experiments were made in which growth hormone was replaced by other lipolytic hormones, ACTH and thyroxine.[6,7] The effect of anti-insulin serum on the development of ketosis was also studied since earlier findings suggested that insulin action might be prolonged in hypophysectomized-pancreatectomized animals and thus prevent development of ketosis.[5] The actions of growth hormone and glucocorticoid were studied also in hypophysectomized rats made diabetic by injection of mannoheptulose. In addition, livers of hypophysectomized rats were perfused with plasma free fatty acids (FFA), at three concen-

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trations, in the presence and absence of growth hormone and glucocorticoid.

METHODS

Hypophysectomized-pancreatectomized rats

Female Charles River rats, fed a commercial stock diet (Purina Laboratory Chow, Ralston Purina Company, St. Louis, Missouri) for one to two weeks and weighing 160 to 180 gm., were used in these studies. The rats were 'totally' pancreatectomized after an overnight fast and then maintained with insulin and tube feeding.[1] By the fourth day they received 12 U. Regular insulin and 4.5 gm. food (Diet 4370[1]) with pancreatin twice daily. On this regimen the pancreatectomized rats excreted less than 3 per cent of the carbohydrate fed and maintained their body weight. The rats were hypophysectomized seven to ten days after pancreatectomy and eighteen hours after injection of insulin and tube feeding.[8] Since their insulin requirement was reduced by hypophysectomy, the rats required only 3 U. insulin with each feeding (4.5 gm. food) for prevention of glycosuria and weight loss. Experiments were performed one week after hypophysectomy. Rats that developed biliary obstruction[1] were discarded from the study.

The experiments were begun in the morning, seventeen hours after injection of insulin and tube feeding. The animals were given no insulin or food during the experiment but were allowed water to drink. On the day after the experiment, the rats were put back on the regimen of 3 U. insulin and 4.5 gm. food twice daily. When rats were used for more than one experiment they were given insulin and food for at least one week between experiments. The rats were not put in the same hormonal treatment group more than once.

The hormones were injected subcutaneously in the back of the animals in a volume of 0.5 ml. The glucocorticoid used was dexamethasone (Merck, Sharpe and Dohme); 5 mg. was dissolved in 100 ml. 2 per cent ethanol in 0.9 per cent NaCl solution and diluted 1 to 10 with 0.9 per cent NaCl solution just prior to use. Lyophilized bovine growth hormone (NIH-GH-B12) was dissolved in slightly alkaline 0.9 per cent NaCl solution just before use. ACTH gel (ACTH-GEL, Armour, Lot D-1001, 40 U./ml.) was used without being diluted. Sodium l-thyroxine (Batch 65, Cal Biochem) was dissolved in 0.9 per cent NaCl solution just before use.

Guinea pig anti-insulin serum (Lot 153, from Dr. Peter Wright, University of Indiana) was fractionated on a Sephadex G-200 column and lyophilized. The gamma globulin fraction, which neutralized in vitro 45 mU. of insulin per milligram of protein, was dissolved in water just before use and injected in a volume of 0.5 ml.

Serial blood samples were taken from the tip of the tail and analyzed for total ketone bodies[9] and for glucose (Glucostat, Worthington Biochemicals).

Hypophysectomized rats

Female Charles River rats (150 to 180 gm.) were hypophysectomized two to four weeks before the experiment and fed stock diet. The rats were fasted overnight and, the next morning, given subcutaneous injections of mannoheptulose (400 mg.) growth hormone (5 mg.) and dexamethasone (2.5 μg.); the mannoheptulose injection was repeated two hours later.[10] Four hours after the injection of hormones, the rats were anesthetized with ether and blood was drawn from the aorta into a heparinized syringe. Plasma was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and aliquots of the solution were taken for determination of total ketone bodies[9] and glucose as above. Aliquots of plasma were taken for the determination of FFA[11] and glycerol.[12]

The parametrial fat pads of these rats were used for in vitro studies of utilization of glucose-U-C-14[13] and release of FFA and glycerol.[14] Incubations were for two hours at 37° in an atmosphere of 95 per cent O_2-5 per cent CO_2. FFA and glycerol in the medium were measured as above.

Liver perfusion

Experiments were performed in perfused livers of hypophysectomized rats, two weeks after operation, fasted overnight. The weight of livers averaged 4.9 gm., or 2.8 per cent of body weight. The livers were perfused in a nonrecirculating system with a 20 per cent suspension of washed beef red blood cells in 4 per cent albumin buffer solution, pH 7.4.[15] The glucose concentration was 8 mM. The FFA concentration of the perfusate was increased by adding FFA prepared from rat adipose tissue triglycerides.[15] Growth hormone and dexamethasone were added to the perfusion fluid as dilutions of the stock solution prepared above. The rate of perfusion was 1.6 $\pm$ 0.05 ml. blood $gm.^{-1} \cdot min.^{-1}$.

Plasma FFA uptake by the liver was determined by multiplying the difference in FFA concentration between the inflow plasma (portal venous) and outflow plasma (hepatic venous) by the plasma flow rate

(blood flow rate times relative plasma volume, 0.83).[14] Acetoacetate, β-hydroxybutyrate, and total ketone bodies were measured by a fluorimetric adaptation[16] of the enzymatic procedure of Williamson et al.[17] The rate of production of ketone bodies was calculated from their concentration in the outflow plasma and the plasma flow rate.

RESULTS

Studies in hypophysectomized-pancreatectomized rats

The effects of dexamethasone and growth hormone, given separately, on blood glucose and ketone body concentrations in hypophysectomized-pancreatectomized rats deprived of food and insulin for seventeen hours, are shown in figure 1. Dexamethasone (2.5 μg.) had no effect on blood ketone body concentration whereas it sustained the hyperglycemia. Injection of 5 mg. of growth hormone increased slowly the blood ketone body concentration, to 40 mg./100 ml. ($P < 0.05$) in twenty-four hours, but did not prevent the fall in blood glucose concentration seen in control rats.

Administration of 0.5 to 1 mg. of growth hormone with 2.5 μg. of dexamethasone induced ketosis; blood ketone body concentration was 30 mg./100 ml. ($P = 0.02$) at four hours, and 80 mg./100 ml. ($P < 0.005$) at twenty-four hours (figure 2). Ketosis did not occur when only 0.1 mg. of growth hormone was given with dexamethasone. Blood glucose concentration at the end

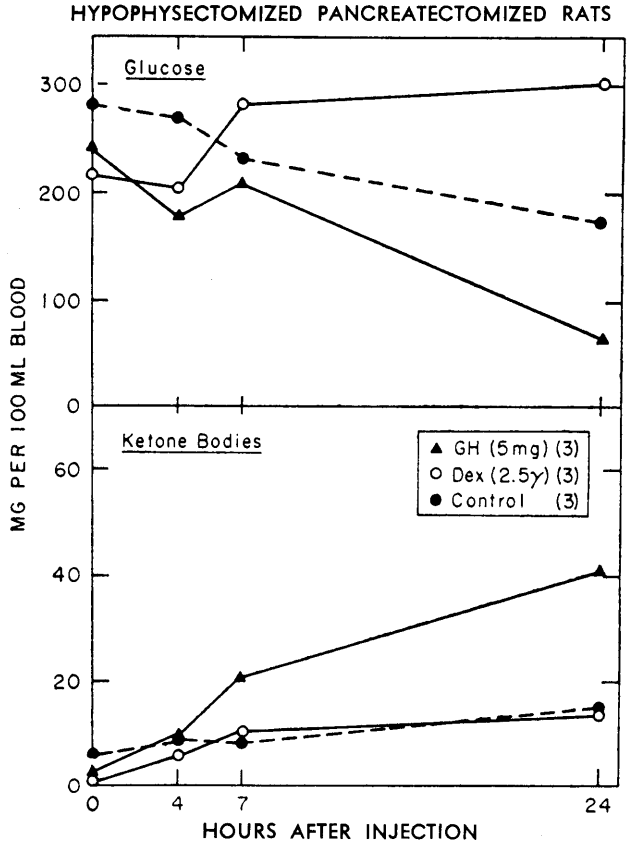

FIG. 1. Effect of growth hormone (GH) and dexamethasone (Dex) given separately on blood glucose and ketone body concentrations in hypophysectomized-pancreatectomized rats. The hormones were injected at the start of the experiment, seventeen hours after the last insulin injection and tube-fed meal. The figures in parentheses indicate the number of rats in each group.

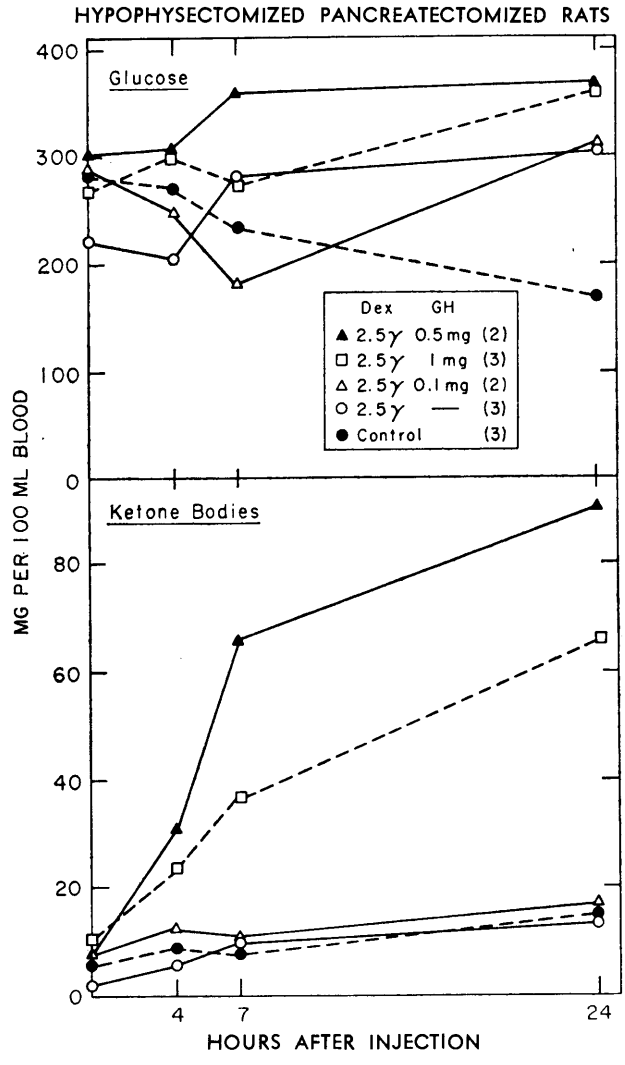

FIG. 2. Effect of dexamethasone (Dex) given separately and with growth hormone (GH) on blood glucose and ketone body concentrations in hypophysectomized-pancreatectomized rats. The hormones were injected at the start of the experiment, seventeen hours after the last insulin injection and tube-fed meal. The figures in parentheses indicate the number of rats in each group.

of twenty-four hours was considerably higher in animals given dexamethasone than in controls.

ACTH (20 U.) increased blood ketone body concentration after a delay of more than four hours, whereas ACTH and dexamethasone (2.5 μg.) given together caused severe ketosis (figure 3). Blood concentration of ketone bodies in doubly treated rats was 30 mg./100 ml. ($P < 0.01$) at four hours and 142 mg./100 ml. ($P < 0.001$) at twenty-four hours. Blood glucose concentration was also higher in doubly treated than in ACTH-treated animals.

The effects of thyroxine on blood ketone body concentration in hypophysectomized-pancreatectomized rats were studied because thyroid secretion is decreased by

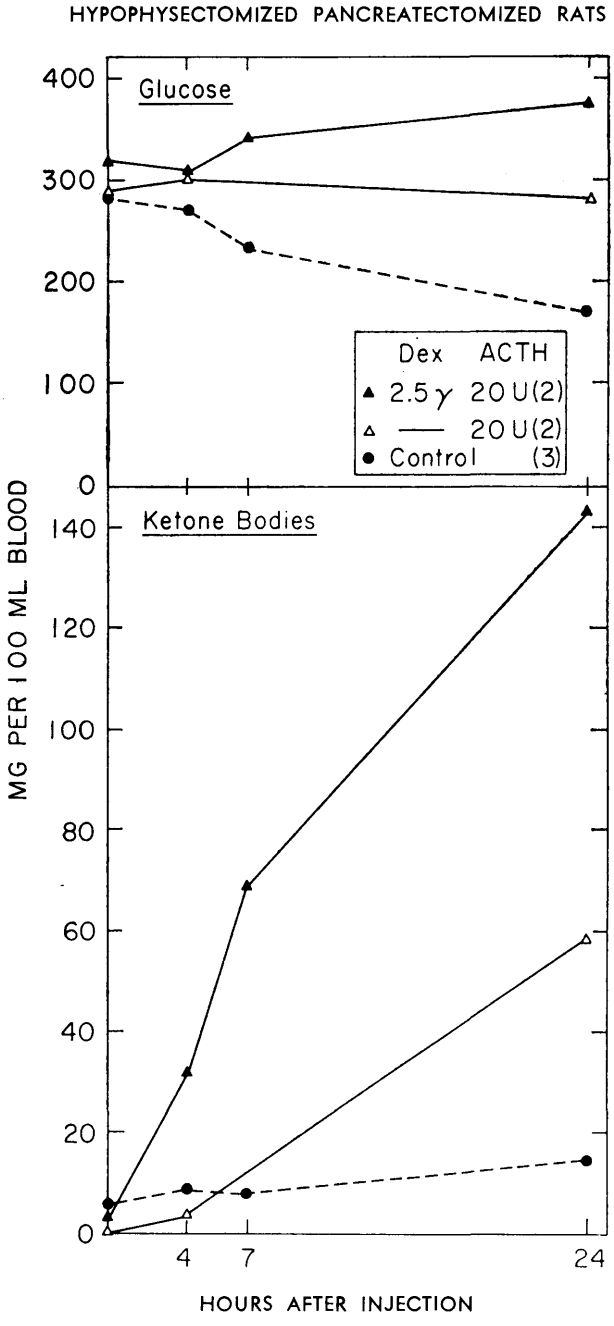

FIG. 3. Effect of ACTH gel given separately or with dexamethasone (Dex) on blood glucose and ketone body concentrations in hypophysectomized-pancreatectomized rats. The hormones were injected at the start of the experiment, seventeen hours after the last insulin injection and tube-fed meal. The figures in parentheses indicate the number of rats in each group.

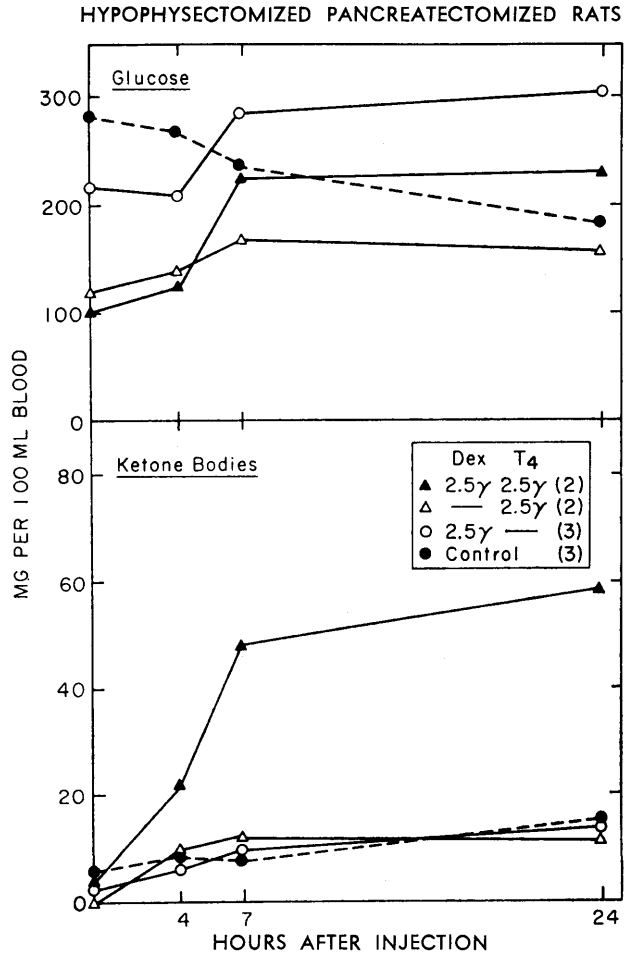

FIG. 4. Effect of thyroxine (T_4) and dexamethasone (Dex) given separately or together on blood glucose and ketone body concentrations in hypophysectomized-pancreatectomized rats. Thyroxine was given daily for two days before the experiment and at the start of the experiment, whereas dexamethasone was given only at the start of the experiment. The experiment was begun seventeen hours after the last insulin injection and tube-fed meal. The figures in parentheses indicate the number of rats in each group.

TABLE 1

Effects of mannoheptulose, growth hormone and dexamethasone in hypophysectomized rats*

Group (n)	Plasma concentration				In vitro adipose tissue			
	Glucose	Ketone bodies	FFA	Glycerol	FFA release†	Glycerol production†	Glucose-U-C-14 to‡ CO_2	Fatty acid
	mg./100 ml.		mM	mM	μmole•gm.$^{-1}$•hr.$^{-1}$		μatom C•gm.$^{-1}$•hr.$^{-1}$	
1. Untreated (6)	99 ±11	6.4 ±0.6	0.35 ±0.05	0.07 ±0.01	1.3 ±0.2	0.45 ±0.06	5.1 ±1.0	2.8 ±0.9
2. GH + Dex (6)	104 ±5	9.3§ ±0.6	0.69§ ±0.08	0.15§ ±0.02	1.4 ±0.1	0.65 ±0.09	3.4 ±0.7	1.2 ±0.3
3. Mh (6)	196§ ±14	9.5§ ±0.6	0.50§ ±0.02	0.17§ ±0.02	1.1 ±0.1	0.35 ±0.04	6.3 ±0.7	3.8 ±0.7
4. Mh + GH + Dex (7)	239‖ ±13	18.0‖ ±1.7	1.0‖ ±0.1	0.25‖ ±0.03	2.4§‖ ±0.3	1.0§‖ ±0.1	2.0‖ ±0.2	0.47‖ ±0.15
5. Mh + GH (8)	155 ±15	13.3 ±1.5	0.80‖ ±0.04	0.19 ±0.02	1.3 ±0.2	0.58‖ ±0.08	4.5 ±0.6	2.5 ±0.5
6. Mh + Dex (6)	335‖ ±35	10.4 ±1.2	0.60 ±0.05	0.17 ±0.02	1.2 ±0.3	0.50 ±0.1	2.8‖ ±0.7	1.1‖ ±0.4

* Rats hypophysectomized for two weeks were fasted overnight and injected twice with mannoheptulose (Mh) (400 mg. every two hours) and once with growth hormone (GH, 5 mg.) and dexamethasone (Dex, 2.5 μg.). They were killed four hours later and plasma and adipose tissue were taken for study. Means ± standard error.

† Fifty to 100 mg. of parametrial adipose tissue were incubated two hours in 4 per cent albumin buffer solution without glucose.

‡ Fifty to 100 mg. of tissue were incubated two hours in 4 per cent albumin buffer solution with 8 mM glucose.

§ Significantly different from untreated control (Group 1) ($P < 0.05$).

‖ Significantly different from mannoheptulose treated (Group 3) ($P < 0.05$).

hypophysectomy. L-thyroxine, 2.5 μg./day, was injected in the morning for two days before the experiment and at the start (zero time) of the experiment. Blood glucose concentration at the start of the experiment was lower in thyroxine-treated than in control rats, whereas blood ketone body concentration was the same in both groups (figure 4). Blood ketone body concentration in the thyroxine-treated group did not differ during the experiment from that in untreated controls, whereas it was increased to 50 mg./100 ml. ($P < 0.01$) in seven hours when dexamethasone (2.5 μg.) was given at zero time to thyroxine-treated rats.

Administration of the gamma globulin fraction of anti-insulin serum, enough to neutralize 2.7 U. of insulin, with dexamethasone (2.5 μg.) had no significant effect on either blood glucose or ketone body concentration.

Lipolysis and ketosis in hypophysectomized rats

The plasma concentrations of glucose, ketone bodies, FFA and glycerol did not change in untreated fasting hypophysectomized rats during the four-hour experimental period (table 1, Group 1). Injection of growth hormone and dexamethasone (5 mg. and 2.5 μg., respectively) increased in fasting hypophysectomized rats the plasma concentrations of ketone bodies, FFA and glycerol without affecting that of glucose (Group 2). Induction of acute diabetes in fasting hypophysectomized rats by injection of mannoheptulose[10] increased the plasma concentrations of glucose, ketone bodies, FFA and glycerol (Group 3). The plasma concentrations of all four constituents were higher when growth hormone and dexamethasone were given with mannoheptulose (Group 4). Growth hormone alone augmented the effect of mannoheptulose on the plasma concentration of ketone bodies and FFA (Group 5), whereas dexamethasone increased the effect on plasma concentration of glucose but not on that of ketone bodies, FFA and glycerol (Group 6).

Parametrial adipose tissues were excised four hours after injection of hormones for the measurement of lipid and glucose metabolism in vitro (table 1). Glycerol and FFA release in tissues from fasted hypophysectomized rats given either growth hormone and dexamethasone (Group 2) or mannoheptulose (Group 3) were not different from that in tissues from untreated controls (Group 1). Tissues from rats given mannoheptulose, growth hormone and dexamethasone (Group 4) manifested increased lipolysis whether compared with those of untreated controls (Group 1) or mannoheptulose-injected rats (Group 3). The tissues of rats given growth hormone and mannoheptulose (Group 5) released somewhat more glycerol than did those of rats given mannoheptulose alone (Group 3). The increased glycerol release was not accompanied, however,

by increased release of FFA. These results are paradoxical in that plasma concentrations of FFA and ketone bodies were elevated in these rats (Group 5), but plasma glycerol was the same as that of the mannoheptulose-treated group (Group 3). Dexamethasone-mannoheptulose treatment did not affect lipolysis in adipose tissue (Group 6 vs. Groups 1 and 3).

The metabolism of glucose by adipose tissue of rats given mannoheptulose and dexamethasone with or without growth hormone (Groups 4 and 6), was significantly ($P < .05$) diminished when compared to that of rats given only mannoheptulose (Group 3).

Effect of growth hormone and dexamethasone on hepatic ketogenesis

Livers of fasting hypophysectomized rats were perfused with a 20 per cent red blood cell suspension in 4 per cent albumin buffer solution containing 0.3, 0.7 or 1.8 mM FFA and 8 mM glucose. The rate of uptake of FFA was directly related to the concentration of FFA in the perfusion fluid (figure 5). The liver removed about 20 per cent of the FFA at the lowest plasma concentration (0.3 mM); about 30 per cent at the concentration of 0.7 mM FFA and about 25 per cent at the highest concentration (1.8 mM FFA). Uptake of FFA by liver usually increased during the first one-half hour and then remained fairly constant during the next three and one-half hours, except when plasma FFA concentration was 1.8 mM. In the latter experiments FFA uptake declined about 25 per cent during the last two hours of the perfusion (figure 5). Addition of growth hormone (1 or 5 μg./ml.) and dexamethasone (0.02 or 0.09 μg./ml.) to the perfusion fluid had no effect on uptake of plasma FFA.

The rate of production of total ketone bodies was directly related to the plasma concentration of FFA and unaffected by addition of growth hormone and dexamethasone to the perfusing fluid (figure 6). The rate of ketogenesis decreased during the four-hour perfusion and reflected the decline in production of β-hydroxybutyrate (figure 7). Formation of acetoacetate tended to increase and, therefore, the ratio of β-hydroxybutyrate to acetoacetate decreased during the perfusion. Addition of growth hormone and dexamethasone to the perfusing fluid affected the production of neither β-hydroxybutyrate nor acetoacetate.

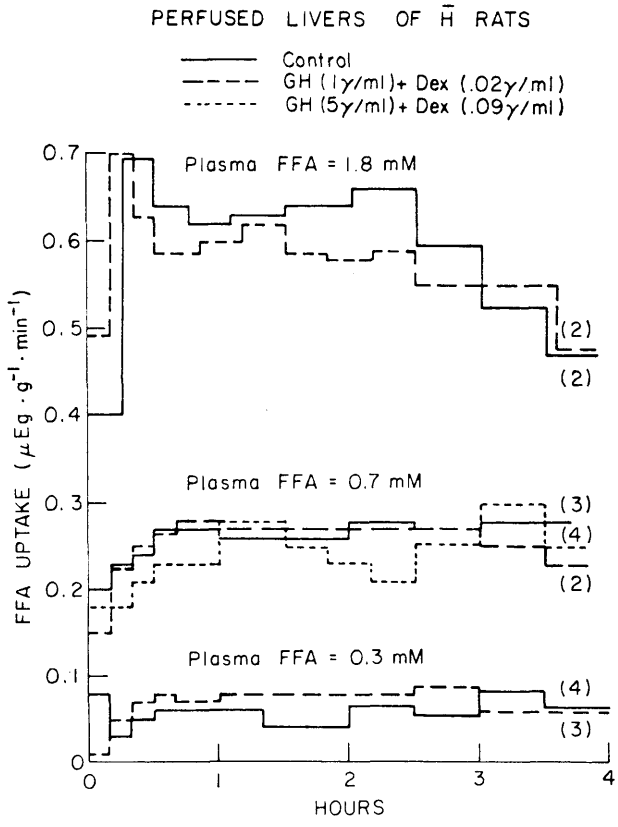

FIG. 5. The effect of plasma concentration and hormones on the uptake of FFA by perfused livers of fasted hypophysectomized rats. Growth hormone (GM) and dexamethasone (Dex) were added at zero time. The glucose concentration of the perfusate was 8 mM. The figures in parentheses are the number of rats in each group.

DISCUSSION

Growth hormone and glucocorticoid given together induced ketosis in pancreatectomized rats deprived of anterior pituitary hormones for one week.[5] The results of the present study suggest that, while the requirement for glucocorticoid may be specific, other hormones, ACTH and thyroxine, may substitute for growth hormone. These hormones have in common with growth hormone, the ability to stimulate lipolysis in adipose tissue.[18] Increased lipolysis per se, however, is not sufficient to increase mobilization of FFA if the metabolism of glucose by the fat cell provides enough α-glycerol-phosphate to re-esterify the additional FFA. Thus, the rate of re-esterification of tissue FFA may limit the release of FFA to the plasma.[18] The failure of growth hormone, thyroxine or ACTH to induce ketosis rapidly when given alone to the hypophysectomized-pancreatectomized rat might be explainable on this basis (figures 1, 3 and 4).

Previously it was reported that glucocorticoid alone produced ketosis in pancreatectomized rats when given one hour after hypophysectomy, whereas growth hormone, alone, was ineffective.[2] From a comparison of

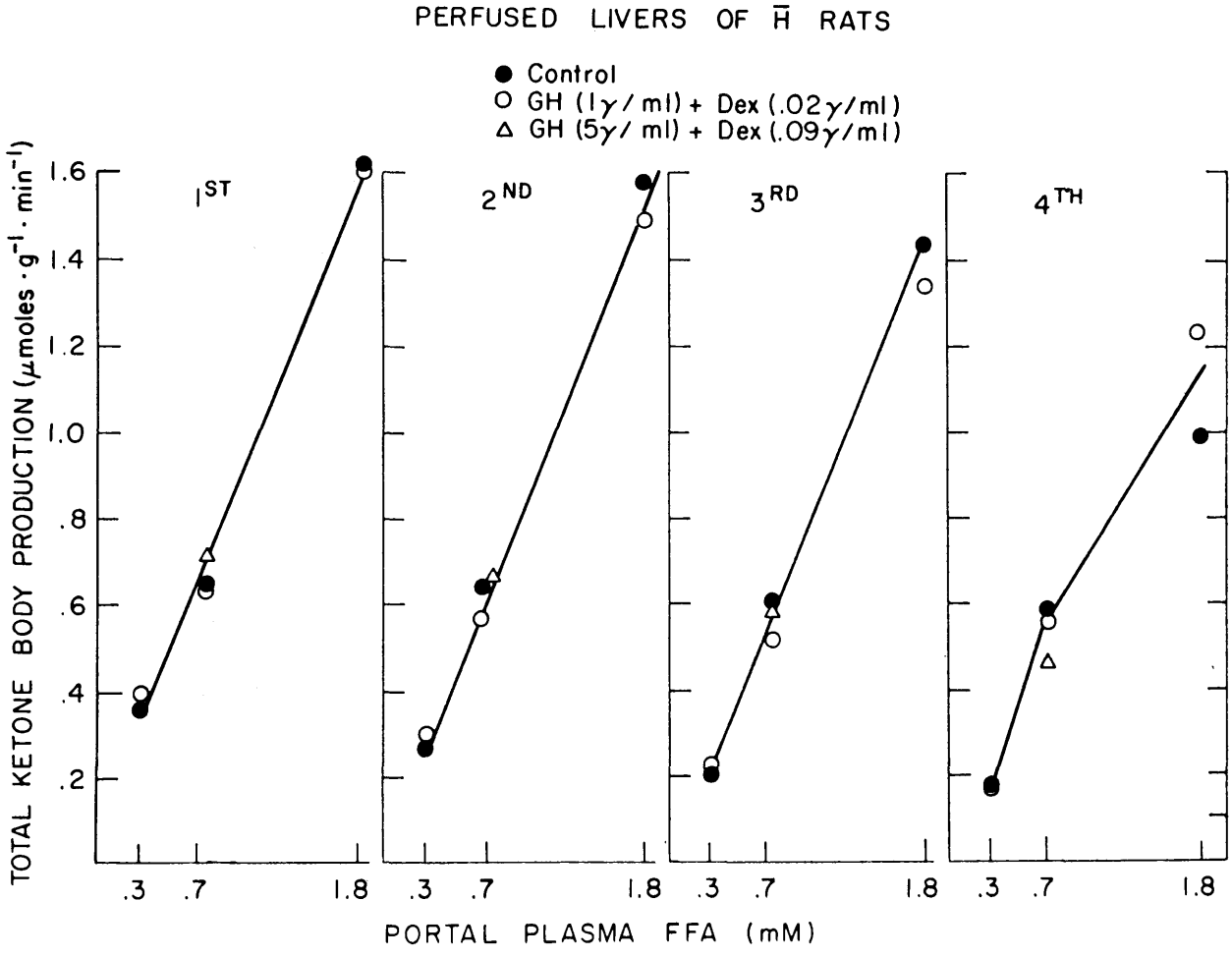

FIG. 6. Relationship between plasma FFA concentration, hormones and the production of total ketone bodies by perfused livers of fasted hypophysectomized rats during four hour-periods. Growth hormone (GH) and dexamethasone (Dex) were added at zero time. Same experiments as in figures 5 and 7.

these results with those in the present study it is concluded that the rate of lipolysis in adipose tissue of diabetic rats one hour after hypophysectomy is sufficient to support FFA mobilization when re-esterification is inhibited by glucocorticoid. But, a week or more after hypophysectomy the rate of lipolysis in adipose tissue is decreased and, both glucocorticoid and a lipolytic hormone are necessary to induce FFA mobilization and ketosis in pancreatectomized rats.

Four processes in the production of ketosis may be amenable to hormonal control: lipolysis in adipose tissue, re-esterification of fatty acids in adipose tissue, hepatic ketogenesis and peripheral utilization of ketone bodies. Only the first three processes were investigated in this study. Administration of growth hormone and dexamethasone to fasted hypophysectomized rats made acutely diabetic by mannoheptulose increased the plasma concentrations of FFA, glycerol and ketone bodies (table 1). The adipose tissue of these rats released more FFA and glycerol and metabolized less glucose, in vitro, than that of control rats. Thus it appeared likely that the important effects of the hormones, growth hormone and glucocorticoid, in the production of ketosis occurred on adipose tissue, increasing lipolysis and decreasing glucose metabolism, with a resulting increase in FFA mobilization.

The results of this study are consistent with observations made of adipose tissue in vitro.[19] Dexamethasone at low concentrations (10^{-8} to 10^{-7} M) increased FFA release and decreased glucose metabolism without augmenting lipolysis in adipose tissue of fasting rats.[13] Growth hormone, at concentrations ineffective when given alone, increased lipolysis and FFA release when added with dexamethasone.[14] These hormonal effects in

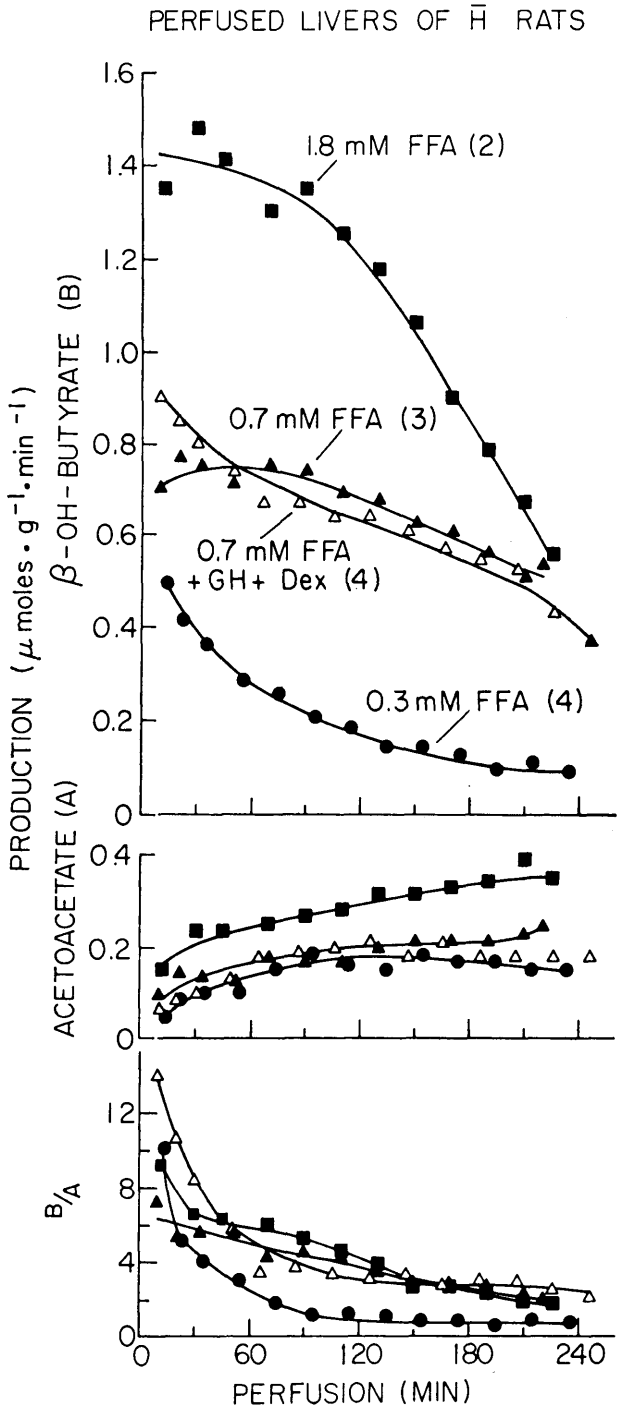

FIG. 7. Effect of plasma FFA concentration and hormones on production of acetoacetate and β-hydroxybutyrate by perfused livers of fasted hypophysectomized rats. Growth hormone (GH) and dexamethasone (Dex) were added at zero time. Figures in parentheses are the number of rats per group. Same experiments as in figures 5 and 6.

vitro are characterized by a lag period of one to two hours during which some limiting protein (enzyme?) is synthesized.[20]

The in vitro metabolism of adipose tissue of fasted intact rats was not altered by treatment in vivo with dexamethasone.[21] However, dexamethasone (10 μg.) decreased the glucose uptake of adipose tissue of fasted adrenalectomized rats. Simultaneous administration of growth hormone and dexamethasone to such rats increased FFA release by adipose tissue.[21] These results, obtained eight hours after injection of hormone, agree with those presented in table 1 in that FFA release by adipose tissue of insulin-deficient hypophysectomized rats was stimulated by treatment with both hormones (Group 4) to a greater degree than by either hormone alone (Groups 5 and 6). Also, glucose metabolism in adipose tissue was depressed in groups given dexamethasone (Groups 4 and 6 vs. Group 3).

The liver is the main source of plasma ketone bodies. FFA released to plasma by adipose tissue are taken up by the liver and oxidized to ketone bodies, mainly β-hydroxybutyrate.[22] Some of the FFA taken up by the liver may be re-esterified to glycerol esters and a small amount completely oxidized to CO_2. Ketosis occurs when ketogenesis is increased and is manifested by hyperketonemia and ketonuria.

Whether liver participates actively or passively in the induction of ketosis was investigated by perfusing livers of fasted hypophysectomized rats with various plasma concentrations of FFA and hormone. In confirmation of results of Clark and Scow,[15] it was found that the rates of FFA uptake and ketogenesis by the livers of fasted hypophysectomized rats were directly related to the concentration of FFA in the plasma. When the FFA uptake by the liver was about 0.25 μEq. • gm.$^{-1}$ • min.$^{-1}$, the ketone body production was about 0.6 μmoles • gm.$^{-1}$ • min.$^{-1}$ during the first two hours of the perfusion (figures 5 and 6). At higher FFA uptake rates, 0.6 μEq. • gm.$^{-1}$ • min.$^{-1}$, the ketone body production rate was about 1.5 to 1.6 μmoles • gm.$^{-1}$ • min.$^{-1}$. Thus at these levels of FFA uptake about one-half to two-thirds of the fatty acid carbon taken up by the liver were released as ketone bodies. However, at low levels of FFA uptake (0.05 μEq. • gm.$^{-1}$ • min.$^{-1}$) the rate of production of ketone bodies exceeded that which could be accounted for by complete conversion of plasma FFA to ketone bodies. In this case endogenous fatty acids, also, were utilized for ketone body production.[3]

Addition of growth hormone and dexamethasone did

not alter the relationship between the plasma concentration of FFA, hepatic uptake of FFA, and ketogenesis (figures 5 through 7). These data are consistent with the views that the principal action of the hormones are directly on adipose tissue and that hepatic ketogenesis is responsive to the level of plasma FFA rather than to dexamethasone and growth hormone per se.

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Correction

The statement on U100 insulin published as a Special Report on page 832 of the July issue of DIABETES referred to the fact that compared unit for unit the same forms of U100, U80 and U40 insulin are equipotent. The word "Lente" was omitted in the example given, which should have stated: "For example, 10 units of U100 Lente insulin lowers blood glucose to the same degree as 10 units of U40 *Lente* insulin, whether administered to patients already being treated with insulin or to patients being treated with insulin for the first time."