Daily Changes in Concentration of Pancreatic and Serum Insulin and of Blood Glucose During 5 Days of Treatment of Rats With Growth Hormone, ACTH, Cortisol, Dexamethasone, and Tolbutamide Alone and in Combinations

Robert W. Bates and Mary M. Garrison

Rats with or without 0.5% tolbutamide in the diet were injected over a 5-day period with growth hormone, ACTH, cortisol, dexamethasone, or various mixtures of these diabetogenic hormones and the daily alterations in blood glucose (BG), serum (SI), and pancreatic insulin (PI) concentrations followed as well as glucosuria. GH (5 mg/day) increased the insulin concentration in the pancreas and serum without affecting blood glucose. ACTH (80 IU/day) decreased PI and increased SI with only a slight increase in BG. Cortisol and dexamethasone decreased PI and increased SI and BG. The severalfold increase in SI with glucocorticoids always preceded the increase in BG by 12-24 hr. Mixtures of GH with the glucocorticoids increased and quickened the rise in BG and SI and the falls in PI, but the rise in SI always preceded the rise in BG by several hours. During multiple hormonal treatment, PI decreased in magnitude usually for 3-4 days but by the fifth day tended to revert toward normal, showing adaptation. In contrast, BG and SI tended to remain elevated and glucosuria persisted. These data suggest that in spite of the high concentrations of diabetogenic agents used compensatory changes were made by the rat with intact pancreas over a period of 5 or more days. This hormonally induced condition of elevated SI and BG but normal PI is characteristic of insulin resistance. Tolbutamide by itself decreased PI without a rise in SI (except for a temporary increase at 4-8 hr after the first injection) and with only a minor decrease in BG. Most of the changes due to the hormones were accentuated in rats with 0.5% tolbutamide in the diet, suggesting that tolbutamide is diabetogenic because it decreases PI.

Previous studies have demonstrated the effectiveness of combinations of growth hormone and glucocorticoids in adequate dosage to induce glucosuria in rats with intact pancreas. These studies also reported the increased insulin levels in the serum and decreased levels in the pancreas after 5 days treatment of rats with and without tolbutamide in the diet. Tolbutamide was shown to be diabetogenic in that (1) it accelerated the appearance of glucosuria, (2) it reduced the threshold dose of hormones to induce glucosuria by a factor of 2, and (3) it decreased the concentration of insulin in the pancreas after 5 days treatment. This is a report of studies to measure the sequence of the changes in the variables of blood glucose (BG), glucosuria, serum insulin (SI), and pancreatic insulin (PI) that occur during 5 days of injection with diabetogen...
genic hormones. Tolbutamide was included because of its apparent diabetogenic action. It was thought that the relative values found might suggest the sequence of events early in the development of hormonal diabetes.

It was earlier demonstrated that the endogenous hormones (ACTH, GH, and prolactin) released from a transplantable pituitary tumor (MtT-F4) caused permanent diabetes in rats with 80% of the pancreas (80% P) removed but not in rats with intact pancreas. Present studies on induction of diabetes by exogenous hormones also have failed to induce permanent diabetes in normal rats with intact pancreas. The same exogenous hormones produced glucosuria but also failed to induce permanent diabetes in rats with 80% P with two or three possible exceptions and then only after 2 wk of injection.

**MATERIALS AND METHODS**

Bovine growth hormone (BGH), approximately 1 IU/mg, was obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases. Cortrophin zinc (Organon) or Acthar gel (Armour) were used as ACTH. Tolbutamide (Orinase) was obtained from the Upjohn Co. Dexamethasone (dexta) (1,4-pregnadien-9a-fluoro-16amethyl-11b,17a,21-triol-3,20-dione) was a gift from Merck Sharp & Dohme by courtesy of Dr. Elmer Alpert. Cortisol was purchased from Mann Research Laboratories. The steroids were injected in solution or as aqueous suspensions of powder (< 40 mesh) in 0.1 N sodium bicarbonate solution containing a trace of a wetting agent. Injections were always subcutaneous every 8 hr for the duration of time selected for each group. Rats were killed 2 hr after the morning injection.

Female rats of the Fischer strain were used. They were 3-6 mo of age and weighed 160-200 g before injection. Rats were treated in groups of four and fed ad lib. They were placed in individual metabolism cages so that food intake, body weight, urine output, and glucosuria could be measured daily. The rats were fed Purina Rat Chow that had been ground to a coarse powder. Powdered tolbutamide was mixed with this diet, usually at a concentration of 0.5%. Thus, a rat eating 10 g of the diet would obtain an oral dose of 50 mg of tolbutamide or about 250 mg/kg. The human dose is about 20 mg/kg/day or about 10%-20% of this dose. The average food intake was usually about 3 g/day. Rats were never fasted, but sometimes some rats on high glucocorticoid treatment refused to eat after 2 or 3 days, but they usually resumed eating by days 4 and 5.

Samples of blood for glucose and insulin determination and of pancreas for insulin determination were taken terminally. Weights of adrenal, kidney, thymus, and liver were also taken to confirm the effectiveness of hormone treatment, but these data will be published separately.

An aliquot (80-150 mg) of pancreas was extracted with 10 ml of 60% ethanol containing 5% NaCl. Insulin was measured in a 1:200 dilution of the pancreas extract and on undiluted serum using microtiter plates in a solid-phase radioimmunoassay method. Blood glucose was measured by a glucose oxidase method. Urinary glucose was measured by titration with quantitative Benedict’s reagent.

Normal values or values on untreated rats vary from experiment to experiment. Table 1 indi-

| Table 1. Reference Values in Normal Rats, Diabetic Rats, and Insulin-resistant Rats |
|-----------------------------|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                            | BG* (mg/100 ml) | SI (µU/ml) | PI (U/g)† | BG/SI | BG/PI | SI/PI |
| Normal                     | 135 ± 6        | 45 ± 5     | 2 ± 0.4   | 3 ± 0.5 | 67.5 ± 7 | 22.5 ± 3       |
| Overt diabetes‡            | 440 ± 26       | 34 ± 12    | 0.40 ± 0.1 | 13      | 1,100 | 85          |
| Insulin resistance§        | 120 ± 17       | 200 ± 25   | 8.0 ± 0.9 | 0.60    | 15    | 25          |

*BG, blood glucose; SI, serum insulin; PI, pancreatic insulin.
†Per gram wet weight of pancreas.
‡Induced by MT-F4 in rats with 80% P.
§Induced by MT-F4 in rats with intact pancreas.
CONCENTRATION CHANGES OF PANCREATIC AND SERUM INSULIN

Cates the normal values and the units of measurement and how the ratios among the three variables are calculated using the values that are observed experimentally. So that whole numbers are usually obtained, the ratios are organized so that the denominator is smaller than the numerator. In clinical studies of diabetics the ratio I/G (insulin index) is sometimes used. This is the reciprocal of BG/SI. Of course, PI values are not obtained in clinical practice. Sample values from a severely diabetic rat are given to show the values of the ratios that are approached in severe diabetes in the rat. Diabetes was induced by hormones from MtT-F4 tumors in 80% pancreactectomized (P) rats. Also given are sample values from rats with MtT-F4 tumors and with intact pancreas that have developed insulin resistance.

A brief statement of the meaning of the values of the ratios based on Table 1 is that in overt diabetes the BG/SI and BG/PI ratios are greatly increased and in the insulin resistant situation they are decreased relative to normal. The ratio SI/PI is not so discriminatory. It may be elevated in overt diabetes, but it may be normal in insulin resistance, if the condition has persisted long enough for compensatory changes to have developed.

RESULTS

Effect of Single Agents

With the single agents, a large dosage, which would cause glucosuria, if possible, was chosen. This dosage was based on previous studies.

Effect of BGH alone (days 1-5) (Fig. 1). Five milligrams of BGH/day did not have any effect on levels of blood glucose, even though the serum insulin level was increased about threefold on days 1 and 2. Serum insulin level then fell back toward normal on days 3-5 but remained elevated. With the increased serum level of insulin at 24 hr there was not a decrease in the pancreatic insulin

Fig. 1. Effect of BGH on blood glucose and insulin levels. Rats were injected three times daily with a total daily dose of 5 mg BGH. Groups of four rats were killed daily. Vertical lines are ±SE.

Fig. 2. Effect of ACTH on blood glucose and insulin levels. Rats were injected three times daily with a total daily dose of 80 IU of ZnACTH. See legend to Fig. 1.
level, which later increased progressively up to the fifth day. The increase in PI was not quite significant (0.1) by day 5. The ratio BG/PI fell nearly to 1 on days 1 and 2 and then returned toward the normal range. There was not any glucosuria. Eighty per cent P rats required 10 mg BGH/day to produce minimal glucosuria; hence, none was expected here with only 5 mg daily.

Increasing concentration of insulin in the pancreas suggests that BGH increases insulin synthesis. The elevated serum level of insulin suggests that an increased release occurs at the same time.

**Effect of ACTH alone (days 1–5) (Fig. 2).** Injection of 80 U of ZnACTH daily in three injections increased the serum insulin level to a maximum of 200 µU/ml on day 4; it then began to decrease on day 5. This high serum insulin level had little effect on the blood glucose except for a suggestive fall on the fifth day. Pancreatic insulin concentration decreased very little to a minimum of 1.4 U/g on day 3 with an insignificant increase on day 5.

The BG/PI ratio fell slowly until it was below 1 on days 3 and 4. This is unlike other hormones and especially combinations, where BG/PI often fell to a minimum by day 1 and had returned to the normal range by day 2 or 3 (except cortisol). BG/PI showed a small rise by day 3 but was normal by day 5. SI/PI reached a maximum of 140 by day 4 but fell to 80 on day 5. These patterns obtained with ACTH were not typical of those obtained with most other hormones. The elevated levels of SI were higher than with BGH and were accompanied by a slight decrease in pancreatic insulin.

There was not any glucosuria. Eighty per cent P rats required 40 U/day of acthar gel to produce glucosuria; hence, none was expected here in rats with intact pancreas. On the fifth day adrenals were enlarged fourfold and thymi weighed 20% of normal.

**Effect of dexamethasone alone (days 1–5) (Fig. 3).** Two-hundred micrograms of dexamethasone daily increased blood glucose slightly on day 1, and by days 3–5 the levels were nearly 300 mg/100 ml. There was not a significant glucosuria until day 4. The serum insulin level was increased three- to fourfold by 24 hr and remained high. The pancreatic insulin level did not fall on days 1 or 2 but on days 3–5 was about 50% of normal, with a very slight suggestion of some recovery on day 5. Again the increase in SI with a decrease of PI suggest a balance favoring release over synthesis.

**Effect of cortisol alone (days 1–5) (Fig. 4).** Fifteen milligrams of cortisol daily duplicated in general the data with dexam. BG increased daily with glucosuria on days 4 and 5. The major differences were that serum insulin rose gradually until day 4 to about 150 µU/ml, which was the same order of magnitude as that reached with dexam. Also, pancreatic insulin fell gradually and was lowest on day 5. These more gradual changes may be due to the insolubility of cortisol, which accumulated from day to day at the sites of injection. The persistency of effect of these depots of cortisol for more than 1 wk after last injection has been reported. Again the increases in SI were accompanied by a decrease of PI.

**Effect of 0.5% tolbutamide in the diet (days 1–5) (Fig. 5).** With 0.5% tolbutamide in the diet, blood glucose levels were significantly decreased at 24 hr from 118 ± 5 at the start to 68 ± 5 on day 1 but increased to the normal range thereafter, showing a physiologic adaptation by 3 days to this effect of tolbutamide.
Fig. 3. Effect of dexamethasone on blood glucose and insulin levels. Rats were injected three times daily with a total daily dose of 200 μg of dexamethasone. See legend to Fig. 1.

Fig. 4. Effect of cortisol on blood glucose and insulin levels. Rats were injected three times daily with a total daily dose of 15 mg of cortisol. See legend to Fig. 1.

Fig. 5. Effect of 0.5% tolbutamide in the diet on blood glucose and insulin levels. See legend to Fig. 1.

Fig. 6. Effect of 0.1% tolbutamide in the diet on blood glucose and insulin levels. See legend to Fig. 1.
Serum insulin levels did not change significantly, nor did BG/SI ratios during days 1 to 5. Pancreatic insulin levels, however, decreased daily from 2.0 ± 0.1 at the start to 0.55 ± 0.01 U/g on day 4 but then increased to 0.86 ± 0.12 U/g on day 5. There was no glucosuria. Hence the main effect of 0.5% tolbutamide in the diet was a steady depletion of insulin from the pancreas with a suggestion of beginning compensation on day 5. These data suggest that 0.5% tolbutamide in the diet produces a decrease in insulin synthesis.

Effect of 0.1% tolbutamide in diet (days 1-5) (Fig. 6). The level of 0.1% tolbutamide in the diet gives a daily intake of about 40 mg/kg, which is twice the usual dose in man. Blood glucose levels were decreased only on day 2. SI showed maxima on days 1 and 4; these maxima were greater than with 0.5%. There were corresponding minima in PI on the days 2 and 4. Both SI and PI were normal on day 5, indicating adaptation. The data were unusually variable.

Effect of 2% tolbutamide in diet (days 2-7) (Fig. 7). With 2% tolbutamide in the diet, blood glucose levels fell to 115 mg/100 ml and remained there for 7 days. PI fell to 0.7 U/g by day 4 but returned to the normal range by day 7. Though BG fell, SI did not increase but fell below normal. This fall in SI was unexpected. It caused a unique increase in BG/SI and little change in SI/PI. The ratios BG/PI were not unusual. The low serum insulin levels suggest an interference with insulin release, but why are the values of BG low and the values of PI low also? The fall in PI could be due to interference with insulin synthesis, but the increase by day 7 suggests that synthesis still does occur after adequate time for compensatory changes. The effects of tolbutamide vary considerably with dosage (Figs. 5-7). Although tolbutamide lowers BG, there is little evidence in these data that the mechanism is via increase in SI. Also, the decrease in PI is suggestive of a diabetogenic effect. The rats tolerate tolbutamide in the diet even at the 2% level.

Effect of 0.5% tolbutamide in diet (0-28 hr) (Fig. 8—open circles). Tolbutamide, 0.5% in the diet, caused a fall in blood sugar level from 141 ± 3 at the start to 102 ± 4 mg/100 ml at 28 hr. Most of this decrease occurred within the first 8 hr. Serum insulin rose from 31 ± 3 at the start to 66 ± 10 μU/ml at 8 hr. This rise was not maintained and fell back to 34 ± 3 μU/ml by 16 hr. Pancreatic insulin levels fluctuated with a slight rise at 4 hr and a general decrease thereafter from a peak of 2.2 ± 0.2 at 4 hr to 1.5 ± 0.13 U/g at 28 hr. In summary, serum insulin levels rose briefly (at 8 hr) but compensatory change had occurred so that normal levels were reached by 16 hr. The fall in BG persisted to 28 hr.

Effect of Multiple Agents

The daily dosages chosen for any hormone were usually less than half those used for the single agents.

Effect of BGH + dexamethasone (0-24 hr) (Fig. 9). Figure 9 shows the changes in blood sugar and insulin levels in the blood and pancreas due to hormonal treatment (BGH, 2 mg and dexta, 50 μg, total dose divided into three injections at 8-hr intervals). Initially there was a fall in blood sugar level of about 20 mg/100 ml within 4 hr; the fall persisted until 16 hr but then returned to the normal level by 24 hr, even though a third injection was given at 16 hr. There
Fig. 7. Effect of 2% tolbutamide in the diet on blood glucose and insulin levels. Groups of four rats were killed on days 2, 4, and 7.

Fig. 8. Effect of 0.5% tolbutamide in the diet with and without injections of BGH + dexa on blood glucose and insulin levels during the first day. Half of the rats were injected every 8 hr until killed, with BGH (0.67 mg) and dexa (17 μg). Open circles are data from rats on tolbutamide only. Filled circles are data from rats that were also injected with BGH + dexa. See legend to Fig. 1.

Fig. 9. Effect of combined injection of BGH + dexa on blood glucose and insulin levels during the first day. Rats were injected every 8 hr until killed, with BGH (0.67 mg) and dexa (17 μg). See legend to Fig. 1.
was not a significant change in the level of insulin in the pancreas. Serum insulin levels had increased by 4 hr and rose steadily to nearly sixfold ($31 \pm 3$ to $175 \pm 7 \, \mu U/ml$) by 24 hr. Hence the major change during the first 24 hr due to hormonal administration was a large rise in serum insulin level.

**Effect of BGH + dexamethasone in rats on 0.5% tolbutamide diet (0–28 hr)** (Fig. 8—closed circles). Injections of the two-hormone combination to rats on the 0.5% tolbutamide diet resulted in a 20% fall in blood sugar over the first 8 hr as for the separate treatment with tolbutamide (Fig. 8) or with BGH + dexam (Fig. 9). However, the diabetogenic effect of the hormones had raised the blood sugar level to normal by 24 hr. Pancreatic insulin levels were lowered significantly by the combined dosages by 28 hr. Serum insulin levels rose steadily to a maximum of $191 \pm 17 \, \mu U/ml$ by 16 hr. This is similar to the effect of the combined hormones without tolbutamide (Fig. 9).

The main difference between the effects of BGH + dexam (Fig. 9) and of BGH + dexam in tolbutamide fed rats (Fig. 8) during the first day was in the extent of the decrease by 24 hr in the level of pancreatic insulin from $1.7 \pm 0.2 \, U/g$ to $1.26 \pm 0.1 \, U/g$ (a tolbutamide effect). BG and SI values were the same in the two cases. By 24 hr the effect of the hormones superceded the effect of tolbutamide on these variables.

**Effect of BGH + dexam with and without tolbutamide (1–5 days)** (Fig. 10).

![Graph](image-url)
The blood glucose level was lower at 1 day and higher at 2 days with tolbutamide than without. By 2 days BG in both groups were over 200 mg/100 ml and, hence, there was glucosuria. BG remained over 250 mg/100 ml for days 3–5. Glucosuria was somewhat higher on days 3–5 in the case of rats on regular diet, probably because their food intake was about 5 g/day whereas in rats on the tolbutamide diet it was only 3.5 g/day. Pancreatic insulin concentration fell to a nadir on day 2 with the tolbutamide diet and day 3 with the regular diet. At all times PI was lower with the tolbutamide diet. The nadir was 0.32 ± 0.04 U/g with tolbutamide diet and 0.49 ± 0.02 U/g with regular diet. In both cases the values were higher by day 5, indicating that the β-cells had developed an insulin producing capacity to compensate for the diabetogenic stimuli. Serum insulin level was somewhat higher at 24 hr than at later times. On the regular diet the serum insulin level fluctuated little during days 2–5. On tolbutamide diet the serum insulin level fell from 150 ± 10 μU/ml on day 1 to 83 ± 6 μU/ml on day 2, followed by a steady daily rise until day 5.

Effect of BGH + cortisol with and without tolbutamide (days 1–5) (Fig. 11).
In this experiment cortisol was used instead of dexamethasone to synergize with BGH. The results are similar in most details to the findings with dexamethasone + BGH in Fig. 10. Minor differences were that the nadirs in pancreatic insulin were 1 day later at days 3 and 4 but with some recovery or adaptation by day 5. Food intake was about 6 g/day on regular diet and 5 g/day on tolbutamide diet. This permitted higher levels of glucosuria, especially on day 3 with tolbutamide, relative to the glucosuria shown with BGH + dexamethasone in Fig. 10.

Effect of BGH + cortisol (days 1–14) (Fig. 12). Because some of the changes induced by the hormones through days 3 or 4 were returning toward normal on day 5, one group of rats was injected daily for 8 days and another for 15 days with a mixture of BGH 2 mg/day and cortisol 6 mg/day. Glucosuria appeared on day 4 and gradually increased until over 4 g was excreted on day 14. Blood glucose was only 260 mg/100 ml on days 8 and 14, down from 300 mg/100 ml; serum insulin had fallen to 80 μU/ml on days 8 and 14 from earlier values of over 100 μU/ml; and pancreatic insulin had increased from a nadir of 0.8 U/g on day 4 to a normal value of 2 U/g on days 8 and 14. The BG/SI ratio, which fell on day 1 and then returned to the normal range for days 2–5, increased to slightly elevated values by days 8 and 14. These higher values were due to the persistently elevated blood glucose levels and a decrease toward normal of the elevated serum insulin levels. The other two ratios rose to 4 days and then fell toward normal by 8 days, primarily because of the changes in the concentration of pancreatic insulin. These data demonstrate the remarkable capacity of the islets of the rats to produce insulin and to counter chronic, strong diabetogenic stimuli.

Effect of three hormones BGH + dexamethasone + ACTH (days 1–5) and of
Fig. 13. Effect of combined injection of BGH + dexta + ACTH on blood glucose and insulin levels. Rats were injected three times daily with a total daily dose of BGH (2 mg), dexta (50 μg), and ZnACTH (40 U). Injections were stopped at 5 days and one group (A1) was not killed until a day later. See legend to Fig. 1.

Stopping injections (1 day after) (Fig. 13). Five groups of rats were injected twice daily with a mixture of BGH-1 mg, dexta-50 μg and ZnACTH-40 U, and one group was killed daily on days 2, 3, 4, and 5. The fifth group was injected for 5 days, as was the group killed on day 5, but was killed 24 hr later without being injected during the last day. Glucosuria and hyperglycemia progressed from days 3 to 5 but fell to preinjection values on day A1, the day after injections were stopped. Similarly, serum insulin fell to nearly normal on A1. In contrast the concentration of insulin in the pancreas rose from 3 on day 5 to 5.5 IU/mg on A1. This is a so-called rebound reaction.

This suggests that the effect of the hormonal stimulus affecting synthesis of insulin persisted longer than that affecting release of insulin. Additional similar time studies after stopping injection are needed.

DISCUSSION

It is well known that BGH, ACTH, dexta, cortisol, and tolbutamide have many effects on tissues other than the β-cells. These effects may modify BG, SI, and PI, but it is beyond the scope of this paper to discuss all of these ramifications.

Without doubt the sequence of changes in BG, SI, and PI are the same or similar during the first 3 or 4 days of stimulation by diabetogenic agents whether an animal eventually becomes permanently diabetic or insulin resistant. Subsequently, if compensatory changes do not overcome the diabetogenic stimulus, PI will continue to decrease until permanent diabetes occurs. If compensatory changes occur which enable the β-cells to survive and make more
insulin, though the diabetogenic stimulus continues, a syndrome like "insulin resistance" follows.

From the data presented above (Figs. 1–13) one is impressed by the daily changes in the interrelations of the three variables studied which reveal the ability of the rat islets to respond to the load imposed by diabetogenic hormones and tolbutamide. A clearer picture of these interrelationships and the significance of the ratios is seen by plotting the variables against each other. In the following figures two additional points are inserted representing extreme yet stable conditions. One point (insulin resistance, Table 1) is that of data from rats with an MtT tumor and an intact pancreas whose islets have been able to compensate for the hormonal stress of continued stimulation imposed by GH and ACTH. Such rats have no glucosuria, normal or slightly low blood glucose, and more than threefold elevated levels of serum and pancreatic insulin. The other point (overt diabetes, Table 1) is that of data from partially pancreatectomized rats with an MtT tumor. These rats had become severely diabetic with glucosuria, high blood glucose, and low serum and pancreatic insulin because the limited amount of pancreas remaining was no longer able to produce sufficient insulin to compete with the diabetogenic hormonal stimuli from the tumor.

Blood Glucose Versus Pancreatic Insulin

Effect of single agents (Fig. 14). BGH increases the concentration of insulin in the pancreas without appreciably altering blood glucose. Tolbutamide does the opposite, decreasing pancreatic insulin markedly. Furthermore, 0.5% tolbutamide lowers blood glucose the first day or two but not thereafter. ACTH caused a gradual decrease of PI for 3 days and remained there with little change in BG.

Fig. 14. Effect of single agents on relative values of BG and SI and their ratio. (Data are from Figs. 1–13.) The numbers by the points designate the number of days injected. The numbers by days 2, 3, and 4 are omitted. The double circle is the value in untreated rats. Filled symbols represent groups of rats that had tolbutamide in the diet. The radiating lines indicate the value of the ratios. The total daily dose is shown. T indicates tolbutamide. Standard errors are omitted but are given in Figs. 1–13.
The diabetogenic steroids appear to increase the blood glucose level only a minor amount, if any, on day 1, but do so progressively thereafter. PI falls after day 1, so that the ratios approach a value of 400 at the dosage levels used. The turning back toward normal on day 4 with dexamethasone and with 0.5% tolbutamide and by day 7 with 2% tolbutamide indicate a successfully increased production of insulin by the islets which are beginning to cope with the demands for insulin. Glucosuria usually occurred with BG >200 mg/100 ml. A severely (permanently) diabetic rat has a BG/PI ratio >1000 due to high BG and low PI values. The MtT rats with intact pancreas that have adapted to the high diabetogenic hormone environment have a value of BG/PI of only 15, which is considerably below normal due to the high concentration of PI presumably induced by high serum levels of GH and ACTH.

Effect of multiple agents (Fig. 15). The BGH (2 mg) and dexamethasone (50 μg) combination caused a large fall in pancreatic insulin on day 1 with a small increase in BG followed by progressive diabetogenic effects for 3 days but recovery of pancreatic insulin concentration began by day 4. The BGH (2 mg) and cortisol (6 mg) did little on day 1 but on days 2, 3, and 4 there were progressive diabetogenic changes in both BG (increase) and PI (decrease). On day 5 there was a major recovery in PI, which then returned to a normal concentration by days 8 and 14 even though there was progressive glucosuria (Fig. 12) and persistent hyperglycemia. Note that lower dosages of the individual hormones were used in the combinations than with the single hormones since their interaction is multiplicative.1

When rats were placed on a 0.5% tolbutamide diet and treated with the same two hormone combinations the large decrease in PI on day 1, due primarily to tolbutamide, was pronounced. There was no increase in BG until day 2. The ratios increased rapidly reaching a maximum by day 2 with BGH + dexamethasone and on day 3 with BGH + cortisol. In both cases PI was increasing toward normal by day 5 with a concomitant decrease in the ratio.

The individual agents at the dosage levels used approached BG/PI ratios of only 200-400 before turning back toward normal (Fig. 14). With the mixtures (Fig. 15) the ratios tended to be higher, especially with tolbutamide, but did not quite reach 1000, nor did they remain that high. In none of the cases was the capacity of the islets to produce insulin exceeded by day 5.
The BG/PI ratio was around 300 before glucosuria appears (BG over 200 mg/100 ml) and is over 1000 in severe diabetes. With the BGH + cortisol combination, glucosuria still continued for 14 days even though the ratio had fallen below 150. Presumably permanent diabetes has supervened when values of PI remain low (0.2–0.3 IU/g) and the pancreas does not recover its ability to produce normal PI values. With BG values > 300 this results in ratios > 1000.

**Serum Insulin Versus Pancreatic Insulin**

**Effect of single agents (Fig. 16).** BGH caused an increase in SI on the first and second day, which was somewhat greater than on days 3–5, when the insulin concentration in the pancreas was increasing (Fig. 16). Tolbutamide (0.5%) caused a decrease in PI without a significant change in the level of serum insulin. The decrease in level of serum insulin with 2% tolbutamide was surprising. In both of these experiments with tolbutamide, the rapid depletion of insulin concentration in the pancreas was not accompanied by an expected rise in level of serum insulin. This suggests that insulin synthesis was stopped and that insulin released from the islets was not replaced. However, this simple explanation may not be adequate when one calculates that the pancreas contains 1000–2000 mU of insulin and only about 0.5 mU is in the circulation.

Both dexa and cortisol induced a significant rise in serum insulin within 24 hr, and this increase was without any decrease in PI. Serum insulin increased further on days 2–3 and then reached a plateau at a level of about 150 μU/ml while PI decreased until day 4 or 5. In the case of dexa there was a fall in the level of SI on day 5. ACTH caused a large rise in SI on day 1, which was maintained through day 5 with only a 25% decrease in PI.

Glucosuria (indicated by an asterisk in Fig. 16) occurred only after a ratio of 100 was reached. An exception to this was the rats given ACTH which had BG values in the normal range with ratios over 100. Also, severely diabetic rats had a ratio of only 85. The SI/PI ratio in rats with MtT after prolonged adaptation to the diabetogenic hormones from the tumor is 25, which is normal. This means that the four- to fivefold increase in SI in the MtT rats has been compensated for by an equivalent increase in PI (Fig. 16, inset). Apparently, physiologic mechanisms, given time (weeks), tend to keep SI/PI constant. This
suggests that SI reflects the concentration of insulin in the pancreas under chronic steady-state conditions.

**Effect of multiple agents (Fig. 17).** In all cases there was more than a doubling of SI in the first 24 hr. In the case of BGH + cortisol there was no concomitant decrease in PI on day 1. Again glucosuria occurred when a ratio of 100 was exceeded, except when this occurred on day 1. However, glucosuria was still progressing in the case of BGH + cortisol on day 14 when the ratio had fallen to a value of 40. Most of the SI values were as high or higher on day 5 than previously. Because the PI values were increased on day 5, a loop pattern was produced in all cases. Tolbutamide tended to increase the SI/PI ratios.

**Blood Glucose Versus Serum Insulin**

**Effect of single agents (Fig. 18).** BGH induced a rise in SI in 24 hr, which was sustained till day 2 and then fell toward normal on days 3–5. There was
not any change in BG. Hence, the BG/SI ratio fell to nearly 1 on days 1 and 2 before returning toward the normal range of 2-4 on days 3-5. Tolbutamide (0.5%) caused BG to decrease on day 1 and slowly returned toward normal by day 5, with little change in SI level. Tolbutamide (2%) caused a fall in SI without a change in BG. ACTH produced the largest increase in SI and without much change in BG. The BG/SI ratio fell progressively to less than 1 on day 4 and was back only to 1 on day 5. Both diabetogenic steroids caused a progressive increase in the levels of both BG and SI. The rise in SI was relatively greater than the rise in BG on day 1 with both and also on day 2 with dexta. The ratios tended to fall on day 1 (except 2% tolbutamide) and returned toward the normal range by day 5.

The BG/SI ratio was 13 in severely diabetic rats. In the nondiabetic rats with MtT and after adaptation, the ratio was 0.6, which is low; this suggests that such a rat is resistant to the effects of insulin. Both BGH and ACTH increased SI with no appreciable change in BG. This rise in SI is obviously by a mechanism different from that in the 4-hr GTT where glucose is the stimulant.

**Effect of multiple agents (Fig. 19).** All combinations produced a marked increase in SI in 24 hr with little or no change in BG. On days 2 and 3, the level of BG then rose to over 200 mg/100 ml with concomitant glucosuria and without a further increase in SI. In short-term studies such as glucose tolerance tests, it is said that SI increases because of hyperglycemia. In contrast, here the diabetogenic hormones have produced a hyperinsulinemia on day 1 without any hyperglycemia. Hyperglycemia in the presence of hyperinsulinemia (insulin resistance) then follows on days 2-5, and this hyperglycemia does not further increase the hyperinsulinemia. This suggests that the diabetogenic hormones may be one cause of insulin resistance. In the case of BGH + cortisol where one group went 14 days, the serum insulin level has definitely decreased. The BG/SI ratios fell typically to about 1 or less on day 1 but had returned nearly to the normal range by day 2.

**Significance of ratios.** The numerical values of the three ratios are not constant; they vary depending on dosage, the duration of exposure to the stim-
ulcus, and the ability of the host tissues to compensate and adapt to the new hormonal environment. This variation with time limits the meaning of the absolute value of ratios at any one time interval to a directional one. The severity of diabetes is indicated by larger than normal values of BG/PI and BG/SI. BG/PI values will be >1000 and BG/SI >10 in permanent diabetes. Insulin resistance is indicated by lower than normal values of BG/PI and BG/SI. BG/PI values can be <50 (due to elevated PI) and of BG/SI <1 (due to elevated SI). The BG/PI ratio approached permanent diabetic levels after 3-4 days when synergistic mixtures were used, but then compensatory changes occurred, primarily an increase in PI (Figs. 14 and 15). The BG/SI ratio usually fell for 1 or 2 days due to relatively high values of SI (Figs. 18 and 19). Later by 4 or 5 days BG had increased in spite of elevated SI and the BG/SI values rose into the normal range. The exogenous hormonal stimuli were insufficient to exhaust pancreatic insulin production, so that SI would fall to a level low enough for BG/SI to increase above 4 toward the severely diabetic range. SI remained high, no doubt because compensatory changes permitted PI production to increase. The daily sequence of changes was reflected also in the SI/PI ratios. Initially the ratio SI/PI tends to increase tenfold with hormonal treatment (Figs. 16 and 17). With pancreatic exhaustion (diabetes), the ratio falls back toward a normal value (Fig. 16) due to a relatively greater decrease in SI.

Curry and Bennett have studied insulin release by the rat pancreas when perfused with a medium containing glucose (300 mg/100 ml). They report that hypophysectomy decreases the insulin released in both the first phase (2-6 min) and in the second phase (30-60 min) of study. In hypophysectomized rats treated for a week prior to perfusion, GH restored only the second phase to a normal level of release, whereas ACTH alone or cortisol alone restored both phases of secretion to the normal level. Calculations from their data indicate that as much as 10% of the insulin in the pancreas was released during the 1 hr of the perfusion. This suggests that turnover rates of insulin are rapid when BG is high, which is the condition in the rats with glucosuria.

A new experimental approach to studies on diabetogenesis that follows changes in PI has been used here. A pancreatic sample requires sacrifice of the animal, which is not practical in man. Chronic diabetogenesis studies require large numbers of animals and large amounts of hormones. For future studies on hormonal induction of diabetes, animals are needed that are not as good at insulin production as the Fischer rat. The 80% P rat is not ideal because of the onerous operation and the still inherent ability of the remaining 20% of the pancreas to make insulin. Variability after partial destruction of β-cells with alloxan or streptozotocin is probably too high. Dogs require only 1%-2% as large a hormonal dosage per kilogram as does the rat to induce diabetogenic effects. Dogs probably have the sensitivity needed but are too expensive for a thorough study. What is needed for further studies is a strain of rats or mice or another small mammal that has a pancreas with limited ability to produce insulin. Some clinical reports suggest that the dose per kilogram to induce elevated blood sugar in man may be as much as ten- to 30-fold less than that in the dog. Hence, studies of the hormonal induction of diabetes may be very important to man.

Many studies by Loubatieres and others have shown that in short-term tests
the acute effect of a single injection of tolbutamide is to increase SI with a concomitant fall in BG. In this paper tolbutamide in the diet also produced a rise in SI at 4 and at 8 hr (Fig. 9). But this rise was temporary and by 16 hr had returned to normal levels, where it remained during 5 days (Fig. 5). At the same time BG decreased by 8 hr (Fig. 8) and remained 10%–20% low for 7 days on 2% tolbutamide (Fig. 7) or for only 2–3 days on 0.5% (Fig. 5) and 0.1% (Fig. 6) tolbutamide. PI did not fall significantly until about 24 hr, but then depletion of PI occurred. The maximum fall in PI with 0.1% tolbutamide was to 1.3 U/g at 4 days; with 0.5% and with 2% it was to 0.7 U/g at 4 days. The major change with tolbutamide is thus a decrease in PI over 4 days, due to an increased insulin release relative to synthesis. This fall in PI with no increase in SI is in the direction of diabetogenesis and makes one wonder about the use of tolbutamide as an antidiabeticogenic agent. But subsequent to day 4, compensatory changes reversed this depletion of PI in the rat (Figs. 5–7). Any explanation for the falls in PI and BG without a corresponding rise in SI (Fig. 5) and an actual decrease in SI with 2% tolbutamide (Fig. 7) must await further studies.

Our data lead one to question the significance of acute 2–4-hr data, such as done in man, for recommending tolbutamide for chronic therapeutic usage. Since the values for BG, SI, and PI alter daily with time (5 days) as compensatory changes occurred, it would seem that recommendations for chronic usage should be based on data obtained after weeks of therapy when any compensatory changes have been established. One recent review of the clinical literature reveals the contradictory nature of reports on the clinical efficacy of tolbutamide.\(^{14}\) Sodoyez and Sodoyez-Goffaux,\(^{15}\) on the basis of daily intraperitoneal injections of tolbutamide (75 mg/kg) into pregnant rats, also have concluded that tolbutamide was without significant β-cytotrophic effect in rats.

REFERENCES


