ASSAY OF A DIABETOGENIC PRINCIPLE IN URINE OF DIABETIC INDIVIDUALS

by

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INTRODUCTION

Primary pancreatic failure is not the only cause of diabetes mellitus.(1) Hyperglycemia and diabetes may theoretically occur as the result of increased insulin destruction, increased insulin requirements (as in obesity), increased inhibition of insulin action as well as decreased insulin production.

Decreased insulin production may be primary, as occurs spontaneously when the pancreas itself is involved in a disease process, or may be secondary to exhaustion following overstimulation by the anterior pituitary. An example of probably secondary islet cell failure is the occurrence of diabetes mellitus in acromegalics, where one could assume the overproduction of a pituitary factor had caused exhaustion of the insulin producing cells. Such secondary islet cell failure has been repeatedly produced experimentally by administration of anterior pituitary extracts to dogs.

An enormous amount of evidence has been accumulated which indicates that the anterior pituitary plays an important role in carbohydrate metabolism. This has given impetus to the search for pituitary factors which may be involved etiologically in human diabetes mellitus. However, despite a variety of approaches and a variety of end points, no definite evidence has been forthcoming that such factors are present in human cases nor has this search produced either reliable concentration methods or reliable assay end points for the detection of a pituitary diabetogenic principle.

It has been our objective to develop concentration and assay methods in order to test whether pituitary diabetogenic factors could be discovered in any cases of clinical diabetes mellitus.

Hypophyseal secretions may affect carbohydrate metabolism either by their actions upon the islets of Langerhans, thus producing alterations in the quantity of insulin elaborated, or may exert their effect by way of other organs and tissues. Examples of extra pancreatic effects of the anterior pituitary upon blood sugar follow:-

The Houssay effect(2) consists of experimentally producing diabetes mellitus by total pancreatectomy and ameliorating the diabetes by hypophysectomy. Conversely, diabetes in pancreatectomized animals can be aggravated by injecting anterior pituitary extracts.

The adrenocorticotrophic hormone effect is more specific in so far as it is known that the pure hormone will increase the production of cortin or "S" hormones elaborated by the adrenal cortex. These "S" hormones increase blood sugar levels by increasing the conversion of amino acids to glucose as well as effecting mobilization of liver glycogen.

The myoglycostatic effect of pituitary extracts is to maintain muscle glycogen independently of the adrenal cortex.(3)

The glycotrophic effect of hypophyseal extracts is to antagonize insulin action peripherally.(4)

Price, Cori and Colowick postulate that a pituitary factor inhibits the hexokinase reaction. The role of insulin is postulated to be the freeing of hexokinase reaction from pituitary inhibition.(5)

It is our intention to deal with the pituitary factors which directly alter insulin production by islet tissue, rather than with the extra-pancreatic pituitary factors. The following summary of the direct effects of anterior pituitary extracts (A.P.E.) upon islet tissue serves the purpose of indicating the specific nature of the effects and also serves to

indicate the nature of the end-point used in our experiments. The effects seem quite characteristic for the diabetogenic or insulinotrophic principle in that a biphasic reaction, increased insulin secretion followed by decreased insulin secretion, is elicited. This biphasic reaction is not mimicked by extrapancreatic hypophyseal effects nor by any currently known effect upon islet cells. (The alloxan hypoglycemia which precedes alloxan hyperglycemia is too transient to be confused with that produced by A.P.H.):-

1. The initial effect of A.P.H. is stimulation of the secretion of insulin.(6,7) This is reflected by an increase in glucose tolerance(8) and is accompanied by usual mitotic activity and hypertrophy of islet cells.(9,10,11)

2. The later effects of A.P.H. consist of continued stimulation of the islet cells to the point of exhaustion. The result is a diminution in insulin output.(10,13) The diminished insulin production is reflected by decrease in glucose tolerance(8) and is accompanied by degranulation and hydropic degeneration of the islet cells.(9,10,12)

3. The final effect of overstimulation with A.P.H. is production of irreparable islet cell damage consisting of hyalinization and atrophy(9,10,12,13) and resulting in permanent diabetes mellitus.(8,9,10,12,13,14,15)

These effects are diagrammed serially in Figure 1. The depiction of stimulation of islet cells by the anterior pituitary gland under normal circumstances is purely conjectural.

Conn and Louis(8) summarize species variability in response to A.P.H. as follows:- "The difference in the final outcome in the dog on the one hand, and in the rat and rabbit on the other, is dependent upon the ability of the islets to keep pace with the massive stimulus, being quickly over-

Figure 1

This schematic representation illustrates the sequence of events resulting from repeated injections of anterior pituitary extract containing the insulinotrophic factor. Note that the effect upon glucose tolerance is biphasic; initial increased glucose tolerance followed by decreased glucose tolerance. These effects on glucose tolerance have served as the assay end points in this investigation.

EFFECT OF ANTERIOR PITUITARY EXTRACT UPON BLOOD SUGAR

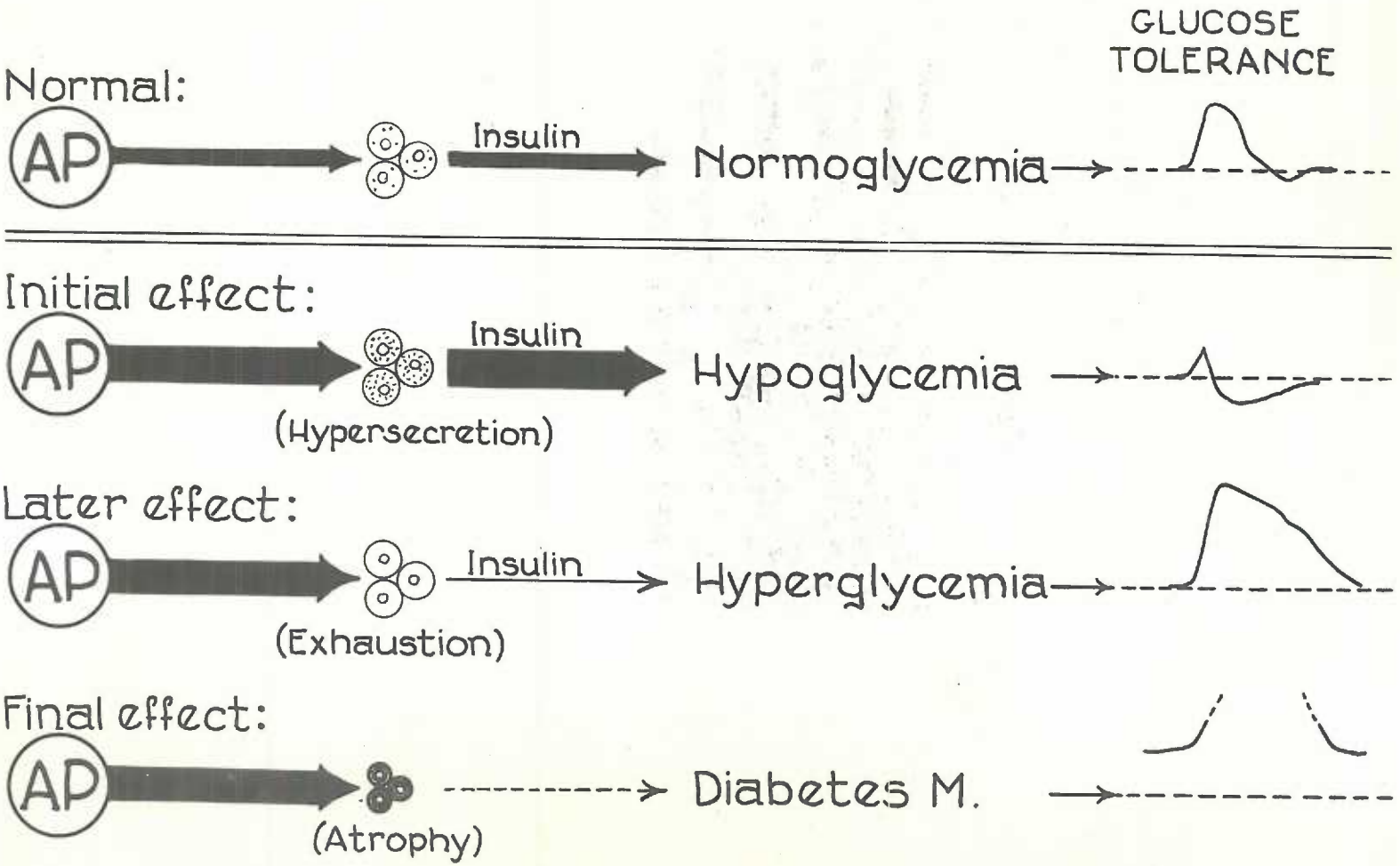

Figure 1

wholmed in the former and being able to respond in the latter. Experience with the rat (intact versus partially depancreatized) indicates that the total amount of islet tissue available to respond is an important factor in the final outcome. Thus three factors appear to determine the result: (1) the size and frequency of the stimulating dose of A.P.E., (2) the total amount of islet tissue available to respond, and (3) possible species differences in the capacity of the islet cells to respond."

Other workers who have searched for a hypophyseal blood-sugar raising factor in blood and urine of diabetic patients have either failed to find such an effect, or such effects as have been found have been non-specific or non-pancreatic.

Himsworth and Kerr(16) after reviewing the attempts to isolate such principles come to the conclusion that concentrates of blood or urine which cause an immediate rise in blood sugar are non-specific and common to the extracts of many tissues. Their own attempts have been directed toward detecting the glycotrophic factor by noting the ability of urine and serum extracts to antagonize the action of insulin. They state: "Tests for the diabetogenic factor were impractical since its demonstration requires quantities far larger than are likely to be found in the blood and urine of diabetic patients."

To achieve our objective of testing the possibility that clinical diabetes could be caused by overproduction of a pituitary hormone, we employed the following three situations or methods:-

1. <u>Type of patient chosen for study</u>. We desired to work with that type of patient most likely to yield a carbohydrate stimulating factor of pituitary origin. An intimate relationship between the hypophysis and the pancreas seems most likely to occur in acromegalics developing diabetes.

Therefore, we chose two acromegalics with clinical diabetes and one with decreased glucose tolerance as subjects.

2. Method of urinary concentration and quantity concentrated. The most desirable method of concentration is one which will efficiently precipitate protein and one which will produce relatively little alteration in protein structure. Therefore, precipitation of the urine with 95% ethyl alcohol at 4°C. was used. Large volumes of urine were used in order to conduct a critical test. From 404 to 900 hours of urine were concentrated per patient and injected into a single dog.

3. End point. The most desirable end point is one which is most distinctive of the pancreatic stimulatory principle of A.P.H. (called either diabetogenic or insulinotrophic hormone). The most distinctive effect of the insulinotrophic A.P.H. principle is the two-phase effect produced in dogs, i.e. initial hypoglycemia followed by hyperglycemia. The biphasic response is detected earliest by observing the response to injected glucose. Therefore, we employed the intravenous glucose tolerance test as our end point. Dogs were chosen as assay animals as they are more sensitive to the insulinotrophic factor than other laboratory animals. Their sensitivity was further increased by decreasing the mass of responsive tissue, i.e. partial pancreatectomy.

Method

Six to eighteen-month old male and female mongrel dogs, maintained on a diet of chopped horse meat, dog chow and water were employed.

All but a segment of pancreas surrounding the pancreatic and common bile ducts was removed surgically, by a method similar to that described by Allen[17] The remaining tissue, about one-fifth of the total, weighed circa 4-6 grams. After clinical recovery from the operation, control glucose tolerance curves were obtained on the dogs. When these remained

constant from one experiment to the next, the injections of extract were begun and intravenous glucose tolerances performed during and after cessation of injections at time intervals indicated on the graphs. Fifty per cent glucose was given intravenously at the end of a 12-15 hour fasting period in amounts of 1.75 gms. of glucose per kilogram of body weight. Immediately prior to running a glucose tolerance test the animal was anaesthetized by administering nembutal intravenously. After obtaining a fasting blood sugar sample, the glucose was given and blood sugar concentrations measured at one-half, one, two and three hour periods. Blood sugars were measured by the method of Nelson(18) employing blood from the femoral vein.

Urine was obtained from the following types of patients (1) two acromegalics with diabetes mellitus; (2) an acromegalic without diabetes but with a decrease in glucose tolerance; (3) a group of patients with idiopathic diabetes mellitus without signs or symptoms of acromegaly (the urine from these patients was pooled and a single determination made).

Twenty-four hour urine collections were made; no preservatives other than refrigeration were used.

Urinary extracts were prepared by a method modified from Keller and Chandler.(19) Five volumes of 95% alcohol were added to one volume of urine. The resulting precipitate was washed with ether and dried, redissolved in a small quantity of water and a volume of 95% alcohol equal to the volume of solution was added. The resultant precipitate was discarded. Two volumes of 95% alcohol were added to one volume of the supernatant fluid. The precipitate was washed three times with ether and stored in the cold until needed for use. Extracts from this were prepared by dissolving the powder in the smallest quantity of water capable of its solution

and filtering off any residue.

The extracts were injected either subcutaneously or intraperitoneally in ascending dosage levels, i.e. a given dose for three days, doubling this dose for the next three days; then tripling the dose for the subsequent three days, and so on until the supply of material was exhausted.

Results

In addition to unoperated uninjected and operated uninjected control dogs, each dog served as his own control since the response to the glucose tolerance test was observed for a minimum of two times after partial pancreatectomy and before urinary extracts were injected.

The partial pancreatectomy affected the glucose tolerance in different dogs to varying degrees, but in no instance was a dog exhibiting frank diabetes employed as an assay animal.

It is to be noted that in no instance did the extracts cause a significant elevation in fasting blood sugar levels.

The effect of urinary extracts from a 38-year old acromegalic woman having a rapidly expanding eosinophilic tumor and insulin resistant diabetes are illustrated in Figure 2. The concentrate of 504 hours output of urine was injected into dog 8A over a period of 9 days, increasing the dosage of extract every 3 days. The most striking alterations in the glucose tolerance tests are noted at one-half and one hours after injecting the glucose. Three days after beginning the injections (Curve 1) there is a drop in blood sugar from 190 mgm.% on the control curve to 100 mgm.% on Curve 1 at the one-half hour level. The blood sugar levels at 1 and 2 hours are also significantly below the control levels. The initial response to the urinary extracts, therefore, is hypoglycemic in nature (increased glucose tolerance). In distinct contrast, the glucose

tolerance test performed 8 days after initiating injections of the extract (Curve 2) exhibits blood sugar levels significantly above Curve 1 and above control levels C and C1. After one-half hour the blood sugar level is 236 mgm.% as contrasted with 100 mgm.% for the 3-day test (Curve 1) and the average of 190 mgm.% for the controls. After one hour Curve 1 is at 36 mgm.% as contrasted with Curve 2 at 140 mgm.%. A test performed one day following cessation of injections (day 10 after beginning) shows a further decrease in glucose tolerance (Curve 3). Maximum hyperglycemia was observed at day 13 (4 days after stopping injections). Curves 5 and 6, performed on days 15 and 21 respectively, continue to demonstrate the hyperglycemic response to the extract although a recession from the maximal response is noted.

It is interesting that this patient's diabetes disappeared entirely for several months following the removal of the eosinophilic tumor.

The second patient with acromegaly and insulin resistant diabetes was a woman 54 year of age. Nine hundred hours of urine were collected, concentrated and injected into dog 8 (Figure 3) during a 9-day period.

The effect upon the glucose tolerance tests in dog 8 are remarkably similar to those of dog 8A in that a definite increase in glucose tolerance was noted 3 days after beginning injections (Curve 1) followed by a decrease in glucose tolerance first noted 6 days after beginning injections (Curve 2). Curves 3 and 4, run after injections were stopped, show continued decreased glucose tolerance whereas Curve 5, run 37 days after initiating injections, approaches the control level.

It is interesting that this patient's diabetes was greatly ameliorated following radiation therapy to the hypophyseal region.

The third acromegalic patient, a woman, age 24, did not have overt diabetes but did exhibit a decreased tolerance to glucose. Four hundred and thirty-two hours of urine concentrate were injected into dog 10A (Figure 4) during a ten-day period.

The glucose tolerance tests performed on the assay dog were remarkably similar to the two preceding experiments. Three days following the initial injection of urinary extract, a clear-cut hypoglycemic response was obtained. Curves 2, 3, 4 and 5, performed 8, 11, 15 and 70 days after beginning the injections, all exhibited a marked hyperglycemic response.

It should be noted that this patient had exhibited feature of acromegaly for a much shorter duration than either of the two patients with clinical diabetes. Her glucose tolerance test, determined several months after an eosinophilic hypophyseal tumor had been removed, was normal.

In summary, in each of the three acromegalic patients tested a biphasic response was elicited; the initial glucose tolerance tests revealing a hypoglycemic response (Curve 1) in each figure), and subsequent tests revealing a hyperglycemic response (Curves 2 to 6 in each figure).

In order to test the validity of the results, three types of control experiments were conducted. The same general plan of performing glucose tolerance tests at similarly spaced intervals was applied in each of the three control situations:

1. Injection of urinary extract from a non-diabetic, non-acromegalic man, 36 years old. Four hundred and thirty-two hours of urine were concentrated and injected into dog 9A (Figure 5) during a 9-day period in the same manner as for the three acromegalic patients. No significant deviations in the glucose tolerance tests were encountered, although Curves 1 and 2, performed 3 and 8 days after beginning injections of extracts, were slightly below the control curves and Curve 3 at 11 days was higher than

Curves 1 and 2.

2. Repeated glucose tolerance tests were performed on uninjected partially depancreatized dogs in the same manner as in the animals that received urine concentrates. Dog 14 and dog 12 (Figures 6 and 7) illustrate the reproducibility of the curves of the glucose tolerance tests. The tests were begun on the 28th and 36th postoperative days, respectively. Note that variations in the curves occur entirely at random and that no biphasic response is elicited. This indicates that partial pancreatectomy results in a reasonably stable preparation and neither a tendency toward hypo or hyperglycemic responses occurs as time after operation elapses. The results also suggest that the repeated performance of glucose tolerance tests at short intervals does not alter carbohydrate metabolism in any consistant manner.

3. Repeated glucose tolerance tests were performed on uninjected, unoperated dogs in the same manner as for operated and injected assay animals. The curves obtained in dogs 1 and 1A are illustrated in Figures 8 and 9. Note that there is somewhat less spread between the curves than for the partially depancreatized uninjected controls.

These three types of control experiments seem to establish the validity of the interpretation that the biphasic response elicited by urine concentrates of the three acromegalics is due to a specific substance contained in the extracts.

An attempt was therefore made to determine whether this specific substance could be detected in the urines of non-acromegalic diabetetics. Since it was possible that only a proportion of diabetics excreted such a substance and since there was no means of predicting which diabetics did so, urine from several hospitalized diabetic patients was collected and

Figure 2

Each successive glucose tolerance curve is labelled. The time before and after injections of urine extracts concentrated from an acromegalic woman with diabetes is indicated in the upper right hand corner. True control curves C and C^1 were performed 11 and 3 days before the injections were begun and Curves 1 and 2 run 3 and 8 days, respectively, after the injections were started.

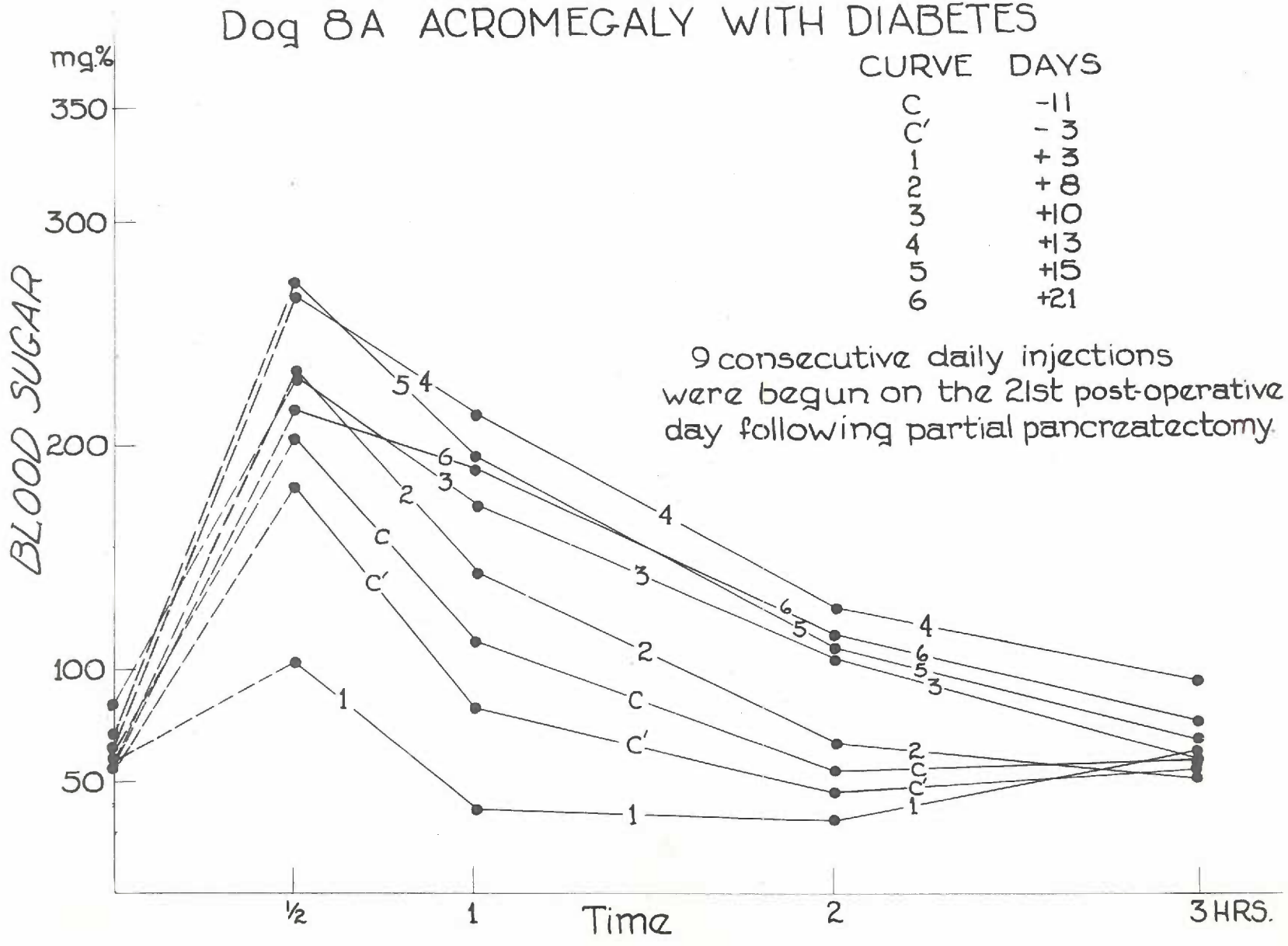
Dog 8A ACROMEGALY WITH DIABETES
CURVE DAYS
C -11
C' - 3
1 + 3
2 + 8
3 +10
4 +13
5 +15
6 +21
9 consecutive daily injections were begun on the 21st post-operative day following partial pancreatectomy.
mg.%
350
300
200
100
50
BLOOD SUGAR
½
1
2
3 HRS.
Time

Figure 3

Successive glucose tolerance curves of a subtotally depancreatized dog injected with urinary extract from a 54-year old woman with acromegaly and severe diabetes.

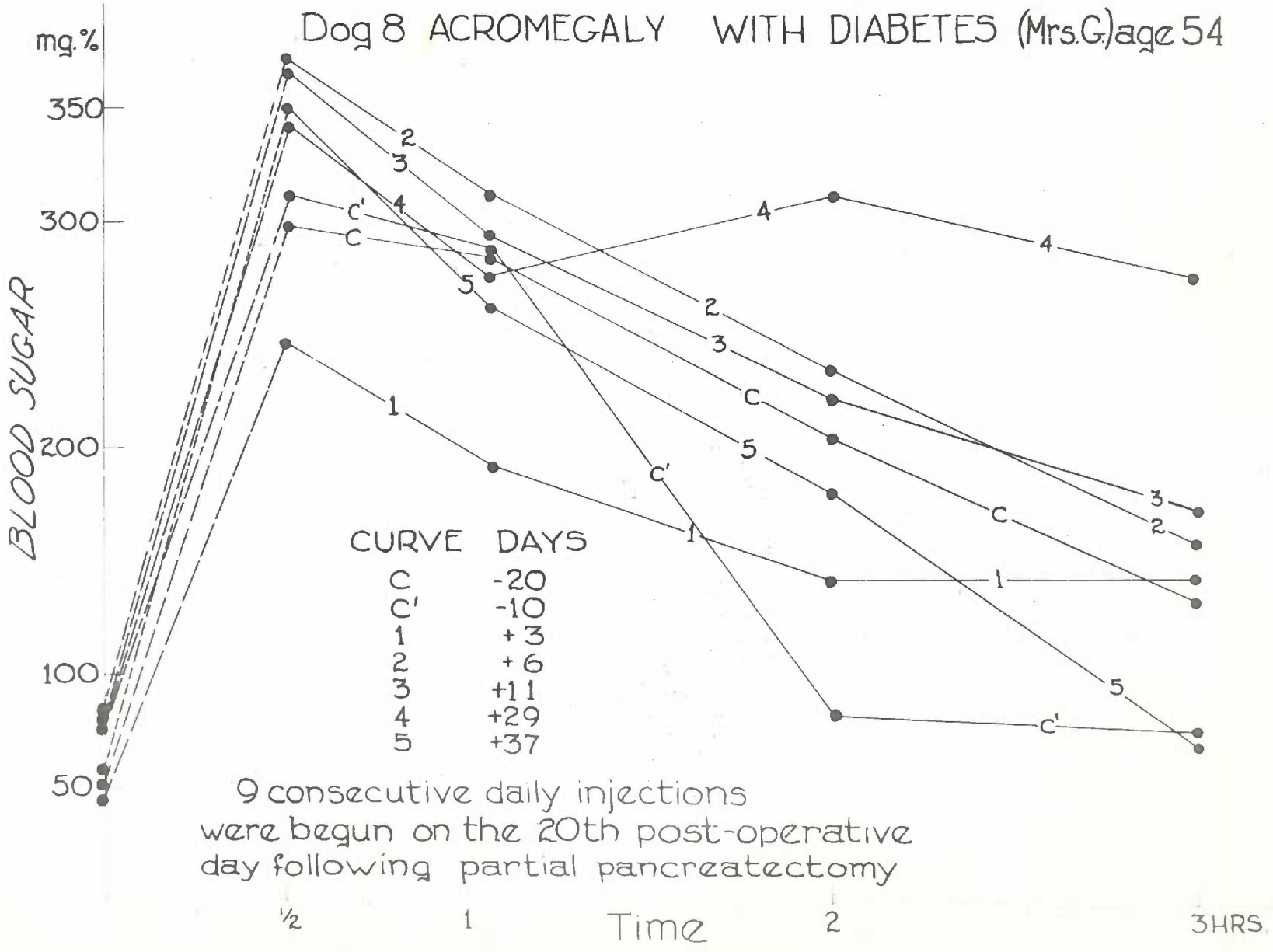
Dog 8 ACROMEGALY WITH DIABETES (Mrs.G.) age 54
mg.%
350
300
200
100
50
BLOOD SUGAR
CURVE DAYS
C -20
C' -10
1 +3
2 +6
3 +11
4 +29
5 +37
9 consecutive daily injections were begun on the 20th post-operative day following partial pancreatectomy
½
1
Time
2
3HRS

Figure 3

Figure 4

Successive glucose tolerance curves of a subtotally depancreatized dog injected with urinary extract of a 24-year old woman who has acromegaly but no overt diabetes.

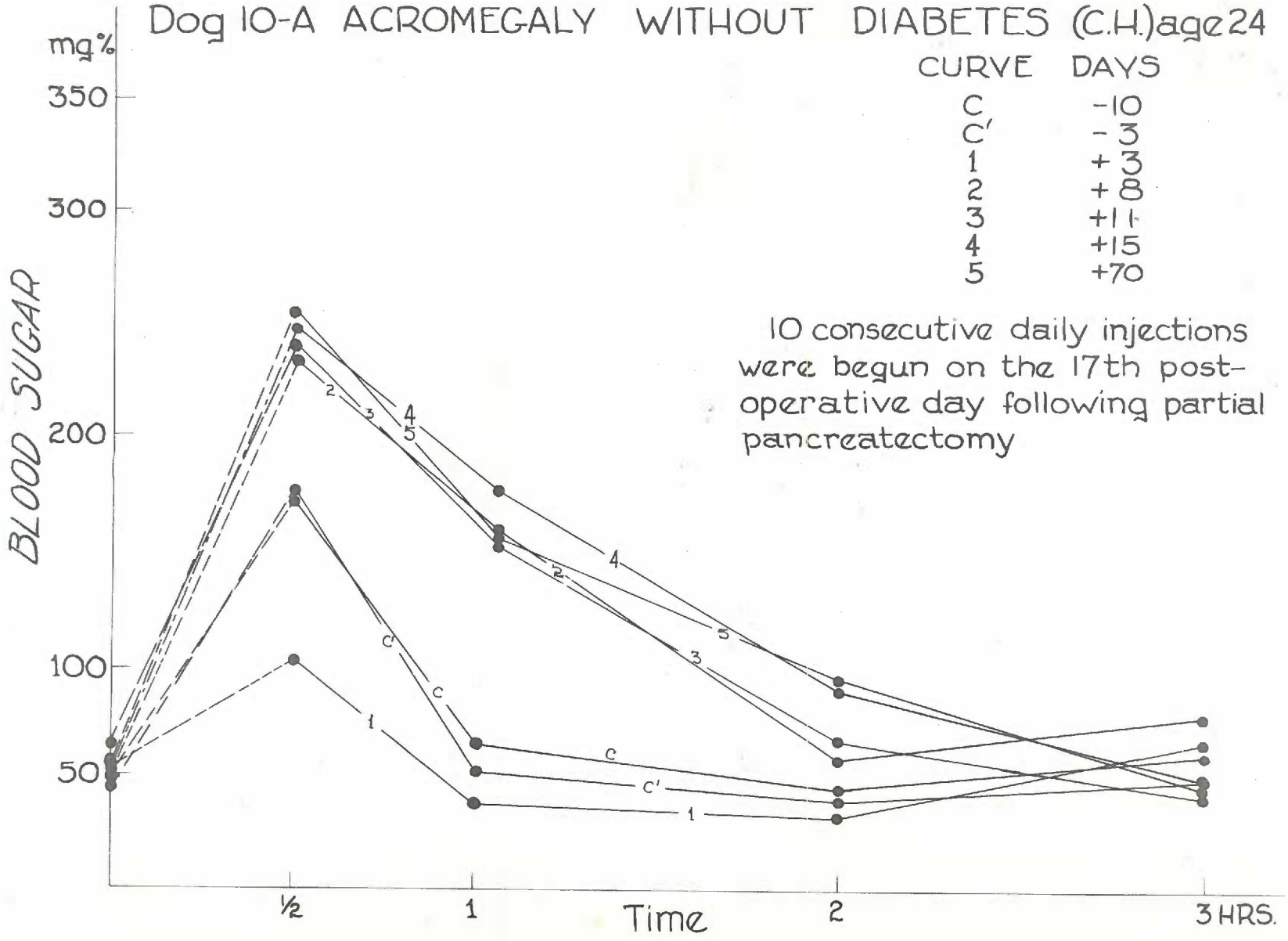
Dog 10-A ACROMEGALY WITHOUT DIABETES (C.H.) age 24
CURVE DAYS
C -10
C' - 3
1 + 3
2 + 8
3 +11
4 +15
5 +70
10 consecutive daily injections were begun on the 17th post-operative day following partial pancreatectomy
mg%
350
300
200
100
50
BLOOD SUGAR
½
1
2
3 HRS.
Time

Figure 4

Figure 5

Successive glucose tolerance curves of an operated dog injected with urinary extract derived from a normal 26-year old male.

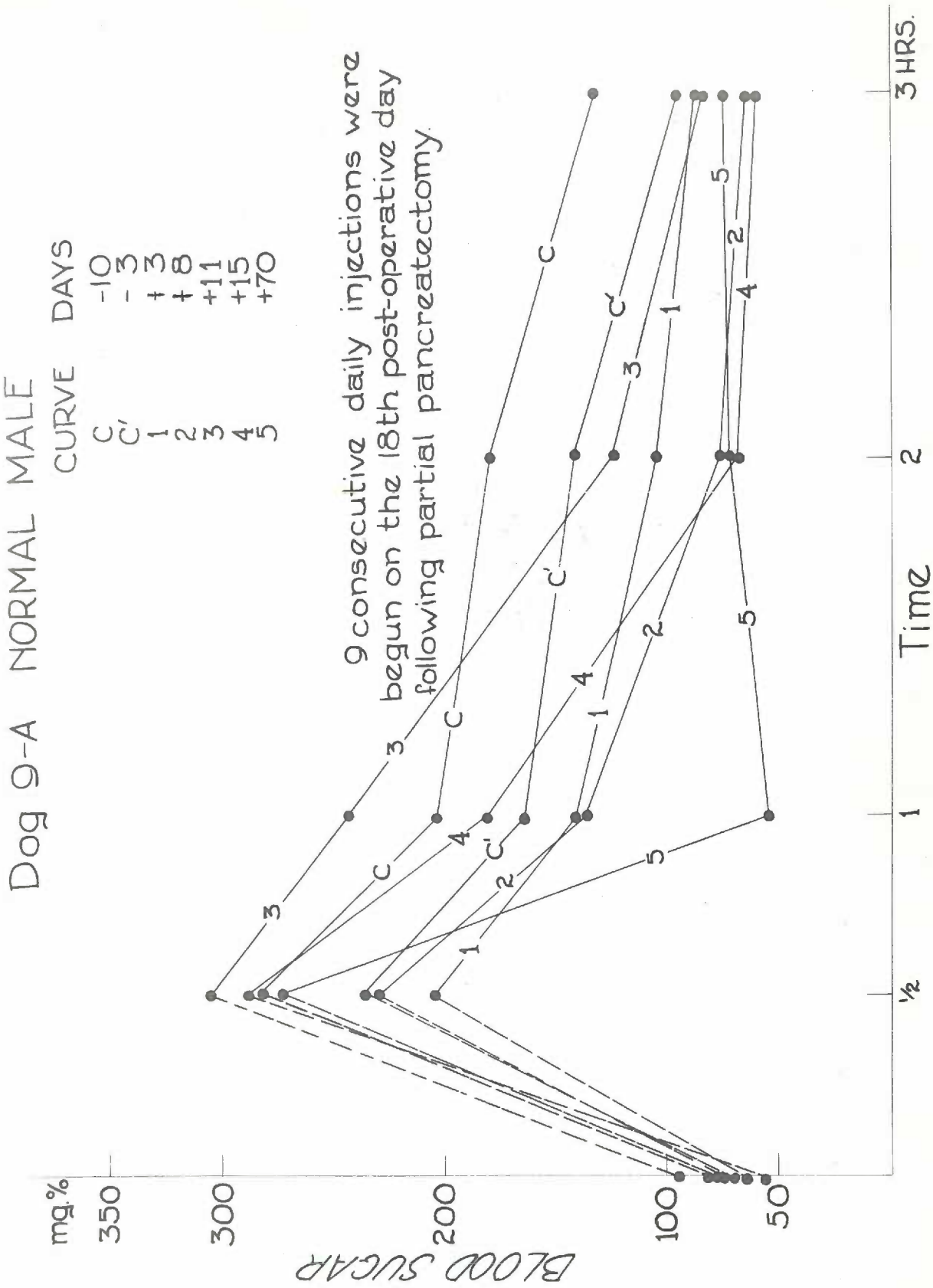
Dog 9-A NORMAL MALE
CURVE DAYS
C -10
C' - 3
1 + 3
2 + 8
3 +11
4 +15
5 +70
9 consecutive daily injections were begun on the 18th post-operative day following partial pancreatectomy.
BLOOD SUGAR
mg.%
350
300
200
100
50
½
1
2
3 HRS.
Time

Figure 5

Figure 6

The response of an uninjected subtotally depancreatized dog to repeated glucose tolerance tests. The successive curves are labelled. The time that each curve was run is indicated in the upper right column. For example, Curve 2 was run 7 days after the first tolerance test (Curve 1). Curve 1 was run on the 28th postoperative day.

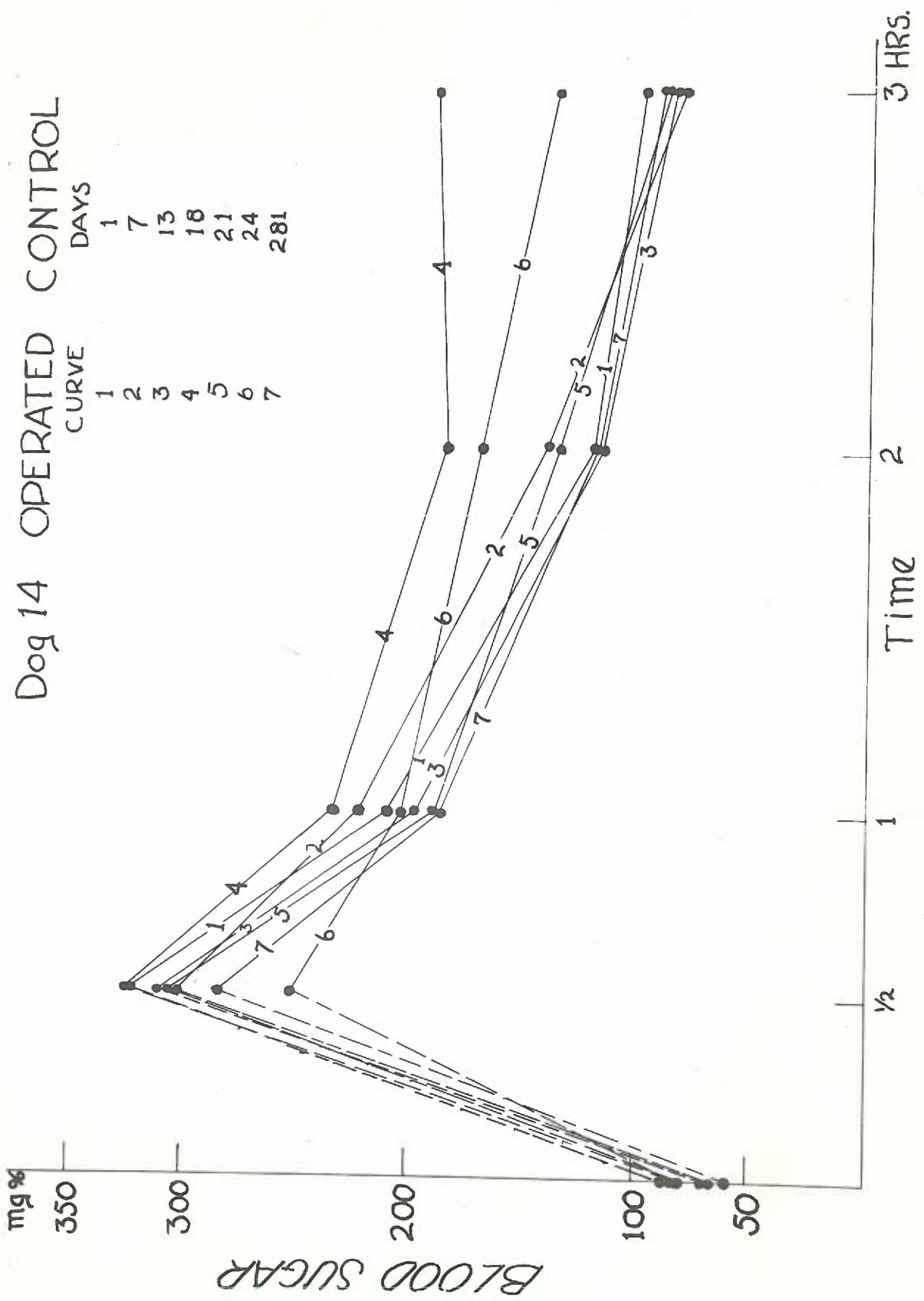
Dog 14 OPERATED CONTROL
CURVE DAYS
1 1
2 7
3 13
4 18
5 21
6 24
7 281
BLOOD SUGAR
mg %
350
300
200
100
50
½
1
2
3 HRS.
Time

Figure 6

Figure 7

The response of an uninjected subtotally depancreatinzed dog to repeated glucose tolerance tests. Curve 1 was run on the 36th postoperative day.

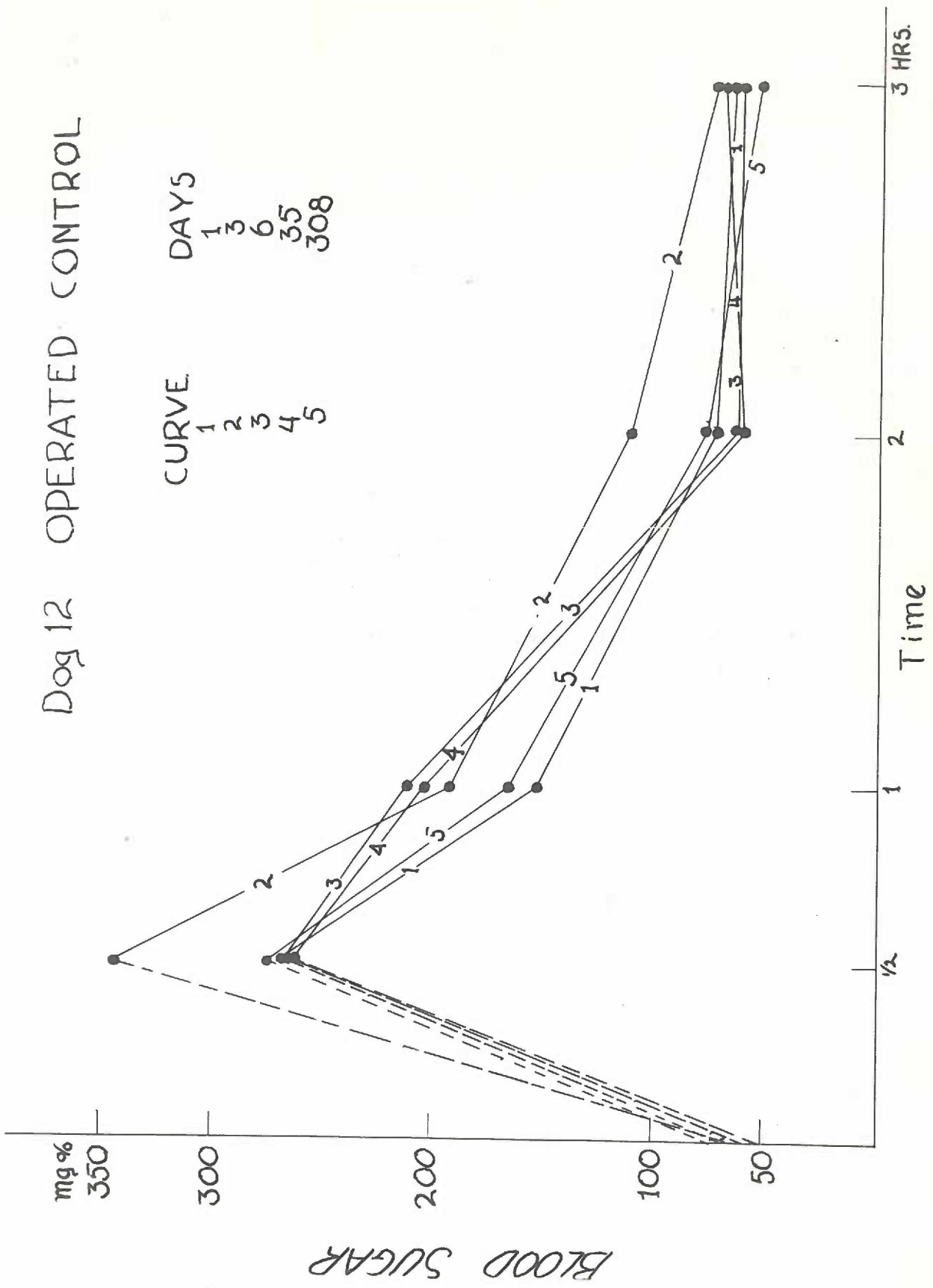
Dog 12 OPERATED CONTROL
CURVE DAYS
1 1
2 3
3 6
4 35
5 308
BLOOD SUGAR
mg %
350
300
200
100
50
½
1
2
3 HRS.
Time

Figure 7

Figure 8

The response of a normal uninjected unoperated dog to glucose tolerance test.

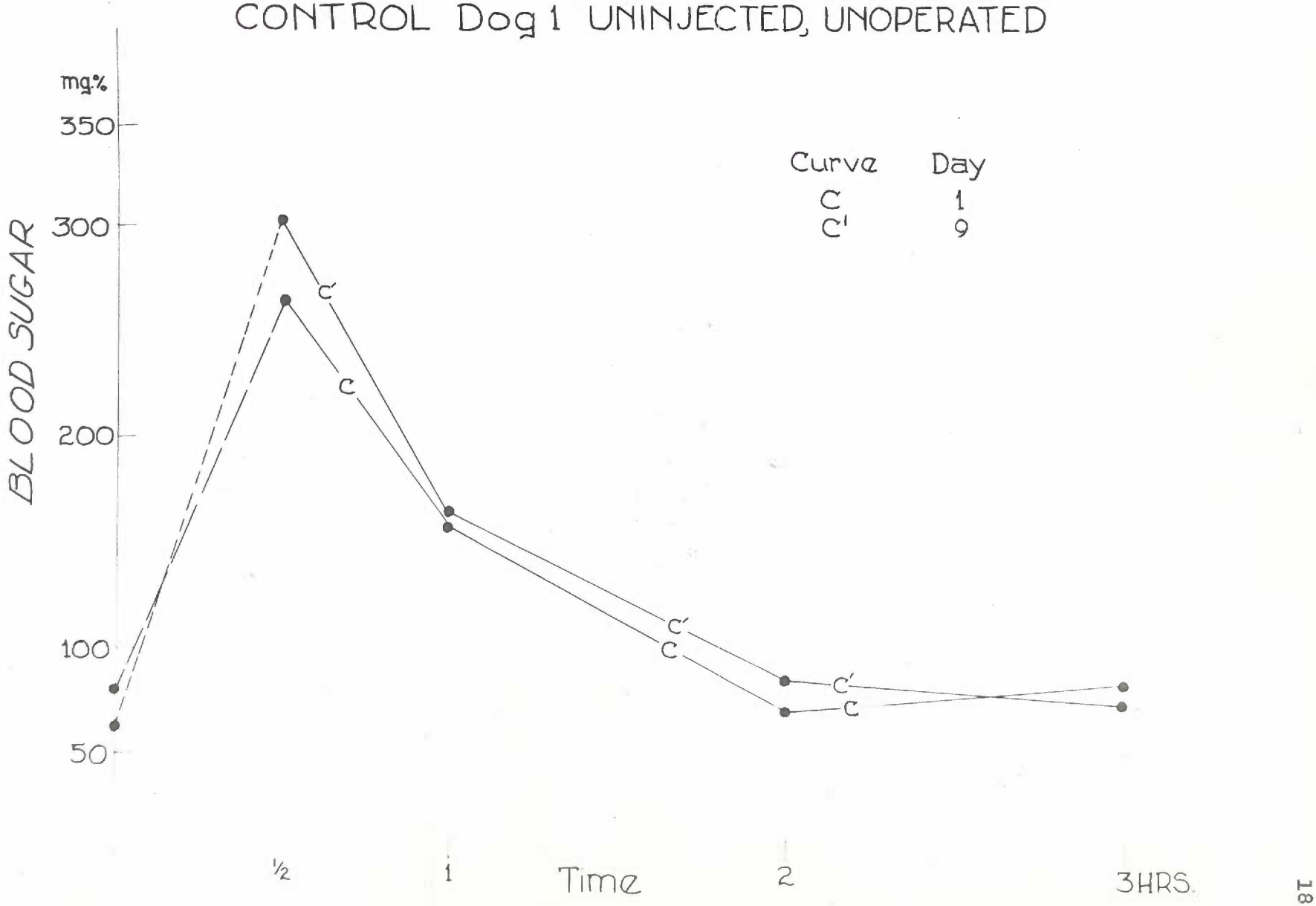
CONTROL Dog 1 UNINJECTED, UNOPERATED
mg.%
350
300
200
100
50
BLOOD SUGAR
Curve Day
C 1
C' 9
C'
C
C'
C
C'
C
½
1
Time
2
3HRS.

Figure 8

Figure 9

The response of a normal uninjected unoperated dog to repeated glucose tolerance tests.

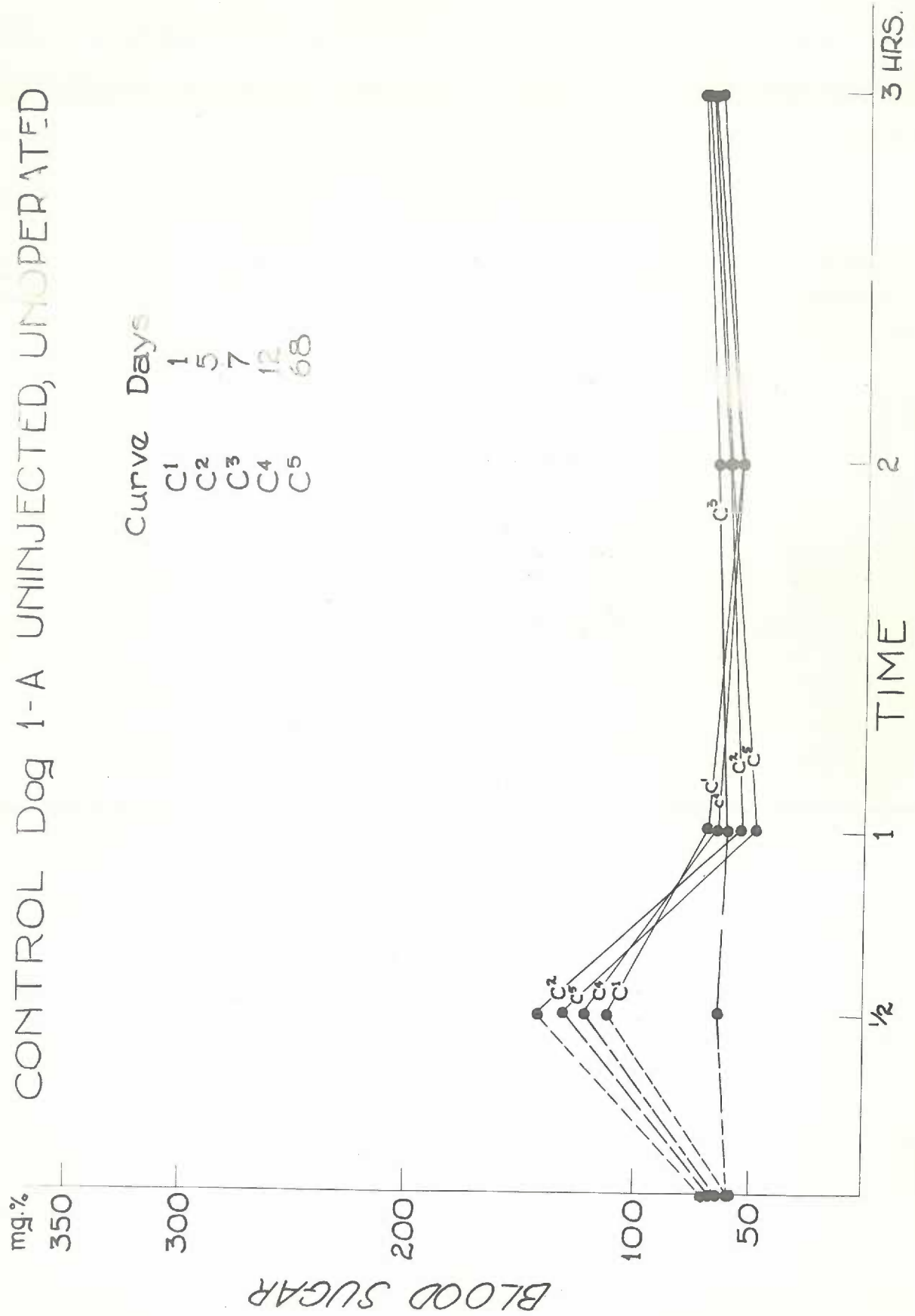
CONTROL Dog 1-A UNINJECTED, UNOPERATED
Curve Days
C1 1
C2 5
C3 7
C4 12
C5 68
BLOOD SUGAR
mg.%
350
300
200
100
50
½
1
2
3 HRS.
TIME

Figure 9

Figure 10

Successive glucose tolerance curves of a subtotally depancreatized dog injected with an extract derived from the pooled urine of several individuals with idiopathic diabetes. The curves are labelled in the same manner as those in Figure 2.

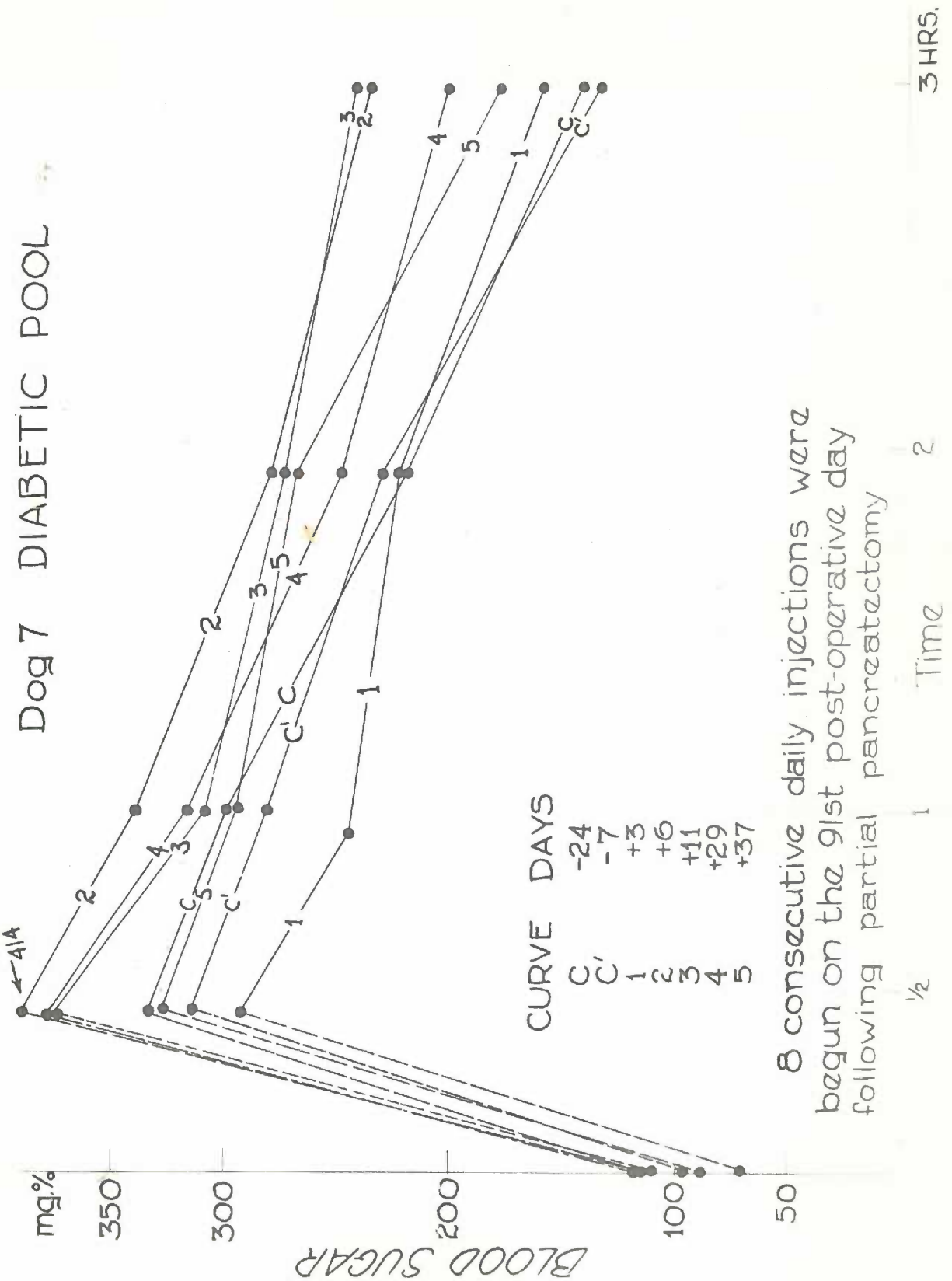
Dog 7 DIABETIC POOL
BLOOD SUGAR
mg.%
350
300
200
100
50
414
CURVE DAYS
C -24
C' -7
1 +3
2 +6
3 +11
4 +29
5 +37
8 consecutive daily injections were begun on the 91st post-operative day following partial pancreatectomy
½
1
2
3 HRS.
Time

Figure 10

pooled. These patients were selected completely at random. Four hundred and four hours of pooled urine was concentrated and injected in the usual manner in dog 7 (Figure 10). The initial glucose tolerance test (Curve 1), performed 3 days after injections were begun, shows slightly increased glucose tolerance as compared to the control curves C and C^1. Curve 2, performed at 6 days, showed a definite decrease in glucose tolerance as compared to Curve 1 and the two control curves. Blood sugar levels at the one-half hour period show a change from 295 mgm.% (Curve 1) to 414 mgm.% (Curve 2). Curves 3 and 4, performed after cessation of injections, continue to exhibit the hyperglycemic response whereas Curve 5 does not.

The similarity between the response of the assay dogs receiving extracts of urine from the three acromegalics and from the diabetic pool are striking and suggest a similar principle was contained in each of these four extracts.

Discussion

Urinary extracts derived from diabetic patients (three acromegalics and pooled urine from several idiopathic diabetics), when injected into partially depancreatized dogs, elicited a biphasic response as determined by the glucose tolerance test. The biphasic response consisted of a hypoglycemic tendency, uniformly detected three days after initiating injections of urinary extracts, followed by a hyperglycemic tendency, detected after 6 to 8 days.

Since this biphasic response has previously been elicited only by material containing the hypophyseal insulinotrophic (diabetogenic) principle, we conclude that the active principle in our extracts most likely is pituitary insulinotrophic factor. None of the pituitary factors that exert an extrapancreatic effect (adrenocorticotrophin, glycotrophic

factor, myoglycostatic factor, etc.) produce such a diphasic response. Non-specific tissue extracts cause an immediate and transient elevation in blood sugar. Alloxan, which produces an immediate hypoglycemic response, seems to be ruled out, since the hypoglycemic tendency was detected 3 days after beginning injections of extract. Alloxan, itself, is of course ruled out as a possible factor since it would be eliminated if present during the extraction procedure.

The pituitary gland is most certainly implicated in the diabetes found in acromegalics since circa 35-40% of all acromegalics have glycosuria(20) and many who do not, have marked deviations in carbohydrate metabolism.(21) In our own cases, surgical removal of the eosinophilic tumor or X-ray therapy to the hypophyseal region markedly improved or abolished the alteration in carbohydrate metabolism.

Therefore, we conclude that the factor dealt with here is most likely insulinotrophic factor of pituitary origin.

We do not, however, conclude that this is the only pituitary factor operating in the diabetes associated with acromegaly. Himsworth and Kerr(16) have clearly demonstrated that the glycotrophic factor was present in the urinary extracts derived from two cases of acromegaly having decreased glucose tolerance curves but were unable to demonstrate this factor in urine from persons with idiopathic diabetes. Fraser, Albright and Smith(21) postulate that the diabetes observed in acromegaly is produced by a pituitary factor and that the disease process recapitulates the reaction seen when pituitary extracts are given to a dog, i.e. initial hypoglycemia followed by hyperglycemia. They arrive at these conclusions from observing the response of acromegalic patients to the insulin and glucose-insulin tolerance tests. They demonstrate both insulin resistance and decreased

glucose tolerance and thus postulate that both the glycotrophic and insulinotrophic factors are increased.

The finding of insulinotrophic factor in the pooled diabetic urine is of exceeding interest. Investigation on individual diabetic patients to determine which excrete and which do not excrete this factor should be given high priority in the hope that therapeutic measures directed at the anterior pituitary will be effective in such cases.

The failure of previous workers to detect the insulinotrophic factor may be ascribed to failure to concentrate sufficient volumes of urine, losses or denaturation during the concentration procedure, and failure to use an end-point specific for the insulinotrophic factor.

Summary

It has been shown that the pituitary has a profound effect on carbohydrate metabolism. It is known experimentally, that large amounts of anterior pituitary extract can produce diabetes in various animals. There is evidence that this diabetogenic action of the pituitary is a direct one, being brought about by the insulinotrophic factor of the pituitary acting directly on the pancreatic islets, causing initial stimulation and later exhaustion.

It is possible a similar process occurs in human diabetes mellitus.

We, therefore, have studied a group of individuals in whom such a factor may be present. These include patients with:

1) acromegaly with frank diabetes

2) acromegaly without frank diabetes, but with decreased glucose tolerance

3) idiopathic diabetes

Search for the factor was made in extracts of alcohol concentrated urine from these individuals. Very large quantities of urine were employed.

Subtotally depancreatized dogs were used as assay animals. Each dog received the full quantity of urine concentrate from each individual.

Changes in glucose tolerance of the dogs were used as the end point.

It was found that the urine of all the acromegalic patients in this study contained a factor which caused an initial hypoglycemic response of the injected dogs to intravenous glucose; later in the course of injections, the same dogs exhibited a hyperglycemic response.

This biphasic effect is exactly analagous to that which might be predicted if anterior pituitary extract containing insulinotrophic factor was injected.

Control dogs, both non-operated and subtotally depancreatized dogs showed no such response to repeated glucose tolerance tests alone, indicating that the biphasic effect is due to a urinary factor.

Urine from a normal individual also failed to produce a biphasic response, showing that the factor is not a non-specific urinary factor.

Pooled urine of patients with idiopathic diabetes, however, caused the same type of biphasic response as found in acromegalic urine.

It is concluded that there exists in the urine of acromegalic patients and in the urine of at least some patients with idiopathic diabetes, a factor which duplicates the action of insulinotrophic factor. It is assumed, therefore, that in all probability the urinary factor is identical with the pituitary insulinotrophic factor.

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