The relationship between the endocrine pancreas in differing functional states and thyroid gland activity in rat and mouse.

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A thesis submitted to the University of Aston in Birmingham for the degree of Doctor of Philosophy.

February 1972

SUMMARY

The relationship between the endocrine pancreas under different functional states, i.e. insulin deficiency, exogenous administration and endogenous beta cell stimulation, and the thyroid gland, both directly and indirectly through the pituitary gland, has been studied in the rat and mouse.

Experiments involving severe alloxan diabetes, partial pancreatectomy and exogenous administration of diketene (aceto-acetic anhydride) have been conducted on female rats in vivo and with glucose and diketene in vitro in female mice. It is concluded that insulin deficiency causes hypo-activity of the thyroid and that the products of hyperketonemia in insulin deficiency are probably the goitrogenic antithyroid agents.

Administration of insulin stimulates thyroid secretory rate in intact female rats. The in vitro studies on female mice failed to demonstrate a similar effect, although there was increased protein binding of iodine in the thyroid gland; this indicates that the effect of insulin on the thyroid is most probably mediated through thyrotrophin.

Tolbutamide and glibenclamide were used to induce increased release of endogenous insulin by the stimulation of beta cells. These failed to give any indication about the effect of endogenous insulin on the thyroid and proved to be antithyroid compounds similar in action to the goitrogenic agents thiourea and thiouracil.

From the conclusion of the above experiments that endocrine pancreas and thyroid gland are not directly related to each other, experiments were conducted to examine the effect of insulin deficiency (in alloxan diabetes) and of exogenous administration of insulin on TSH levels in the blood of intact female rats using a modified method of Brown and Munro (281). It was found that both insulin deficiency and excess insulin elevate TSH levels. Probable explanations for these phenomena have been discussed.

CONTENTS

ACKNOWLEDGEMENTS

I am greatly indebted to Professor A.J.Matty for the suggestion of the topic, recommendations for financial grants and continuous encouragement and guidance in the production of this thesis.

The financial grant from Hoeschst Pharmaceuticals and fee award from the British Council are gratefully acknowledged.

GENERAL INTRODUCTION

One of the major functions of the endocrine system is to maintain the constancy of the milieu interieur. A perturbation, such as removal or malfunction of one of the glands leads to changes in the function of many of the other glands and/or a change in the expression of the activities of other hormones upon target cells. Beta cells, part of the endocrine pancreas produce a hormone called insulin (1), which plays a leading role in carbohydrate metabolism and exerts an influence on fat and protein metabolism by both direct or indirect action (2) and creates much interaction between pancreatic and other endocrine secretions. Decrease in blood-sugar follows adrenalectomy in rats (3) and hypophysectomy has been found to bring about an increase in the loss of carbohydrate (4). Also amelioration of diabetes mellitus has been shown in the depancreatized dog following hypophysectomy (5). Furthermore in diabetic animals, the blood-sugar level, excretion of carbohydrates and rate of ketosis are reduced following hypophysectomy (4,5,6,7,8,9,10) and the animals show an increased sensitivity to insulin.

Fractions of the anterior pituitary gland and the purified pituitary hormones, growth hormone and corticotrophin are diabetogenic in many animals in that they induce β-cell exhaustion (11,12, 13,14,15). Insulin treatment does not increase the body weight or composition of hypophysectomized-force fed rats (16). However, adrenal cortical extracts and corticoids administered to depancreat-

ized-adrenalectomized rats increase the severity of diabetic symptoms (17). Evidence of sexual dimorphism has been found regarding the severity of diabetes in male and female rats (18,8) and after subtotal pancreatectomy, the incidence of diabetes is greater in male than female rats and androgen administration increases the severity of the diabetic response. Hormone interaction is thus well documented.

If the interaction of the thyroid and the endocrine pancreatic gland is considered there is ample evidence that thyroid hormones affect virtually all aspects of carbohydrate metabolism. Many of their influences are dependent upon or modified by other hormones, in particular the catecholamines and insulin. Thyroid hormone administration reduces the incidence of diabetes following extensive partial pancreatectomy and leads to enlargement of islets and new islet formation (19). The administration of sufficiently large amounts of dessicated thyroid gland to intact and hypophysectomized rats results in increase of islets of Langerhans and also in the islet weight per unit body weight (20). Degranulation of islet cells and increase in β and α cells has been reported to result from thyroxine administration (21). In thyrotoxicosis in man the hypoglycaemia response to insulin is increased (22). In thyroxine injected rats, adipose tissue doubles its usual rate of uptake of glucose and production of CO_2 at the optimal *in vitro* dose of insulin and has a greater dose response to optimal insulin concentration (23). Uptake of glucose *in vitro* is increased in the rat by previous *in vivo* injections of triiodothyronine, which also increases diaphragm glycogen deposition after exposure to insulin *in vitro* (24). In animals with intact and healthy pancreas, thyroid hormone administr-

ation does not produce diabetes but transient glycosuria appears in man undergoing thyroid treatment (25,26,27). Daily administration of thyroid hormone produces diabetes when the resistance of pancreas has been previously diminished by drugs (28) or by resection of eighty to eighty-seven per cent of mass of pancreas (29). This thyroid diabetes is transitory and disappears a few days after the administration of thyroid hormone is discontinued (25,27,30,31,32). After a prolonged thyroid hormone treatment, dogs remain diabetic. Even after suspension of the treatment, the pancreas failed to secrete any more insulin (25,27,31,32). After radio-iodothyroidectomy, there is a slight and transitory diminution of glycaemia and requirement of insulin in the dog (33). In rats, surgical thyroidectomy or radio-iodothyroidectomy markedly diminishes the incidence of alloxan diabetes and in a large number of cases prevents the appearance of diabetes after 95% pancreatectomy (26). Radio-iodothyroidectomy produces the regression of diabetes in many cases in alloxan treated rats (34).

This brief literature review shows that most of the literature has dealt with thyroid effect on the endocrine pancreatic activity in the thyroid-pancreatic interaction. There is, however, a certain amount of literature concerning the way in which the endocrine pancreas affects the thyroid gland activity. Alloxan treated rats show increased colloid and decreased cell height in the thyroids (35), reduced uptake of ^{131}I and subnormal thyroid hormone release rate (36) and decreased thyroid secretory rate (37). Administration of increasing levels of insulin increases the thyroid secretory rate in normal rats (38). The defects of the diabetic-subnormal thyroid

secretory rate have been corrected by insulin administration (37,39). Insulin enhances the ability to incorporate ^{131}I into protein bound fractions in the cultured foetal thyroid gland (40,41). In man, it stimulates thyroid function (42) by increasing the discharge of iodine hormone (43) and in diabetics insulin therapy increases the daily consumption of thyroxine (44).

There are some recent investigations which could be attributed to indirect effect of the endocrine pancreas on thyroid gland activity. These are that degranulation of acidophilic cells and decreased basophilic cell counts have been found in the pituitaries of alloxan diabetic rats (45), that high TSH values have been found in juvenile diabetics and patients with advancing retinopathy (46,47).

The thyroid gland has a high concentration gradient for iodine, the iodide from the blood plasma is collected by the epithelial cells (48,49), under aerobic conditions (50,51,52). Iodide is oxidised by an enzyme "tyrosine iodinase", which transfers iodine to available tyrosine in the follicles. It has been shown that from the first hour after the administration of ^{131}I all the uptaken iodine is present in the colloid both in the rat (53) and the mouse (54). The free iodine is organically bound to tyrosine, as the surviving thyroid slices have been found to attach radioactive iodide in organic form in rat (55) and man (56). Of the various hypothesis, the one proposed by Harlington (57) that tetra-iodothyronine or thyroxine results from condensation of two molecules of diiodotyrosine seems most satisfactory. These iodoproteins are the biological reserve of hormones (58) and thyroid proteolysis leading to diffusible products of low molecular weight takes place in the colloid vesicles and is carried out by a proteinase system of a cathaptase nature (59).

It has been found that TSH activates cathaptase *in vivo* (60) and *in vitro* (61); it liberates the iodothyronines from thyroglobulin. There is a form of physiological selection operating and it has been found that mainly thyroxine and triiodothyronine go into the circulation (62). The hypothalamus establishes the set point of response of the pituitary to changes in blood level of thyroid hormone (63). *In vitro* TRF (thyrotrophin releasing factor) increases both thyrotrophin synthesis and release by the mouse pituitary (64). *In vivo* injection of the factor results within five minutes in a depletion of pituitary thyrotrophin content (65). Treatment with thyroxine does not alter hypothalamic TRF (66); the block is overcome by actinomycin or cycloheximide, which suggests that TRF does not act through acute synthesis of protein, but that thyroxine may cause synthesis at the pituitary level of a labile substance that inhibits TRF effect (67).

Iodide "pick up" by the gland is a mechanism independent of its storage (68). The thyroid iodide concentration depends upon many factors such as serum iodide level (69,70,71) and iodine content of the diet (72,73,74). The anterior pituitary and thyroid comprises an autonomous unit that maintains a basal level of thyroid activity in the classical feedback mechanism (75,76). An anterior basal hypothalamic factor, transported to anterior pituitary via hypophyseal portal vessels, tonically stimulates TSH release to increase thyroid function above the basal level. Hypothalamus filters the portal blood, thereby altering its thyroid hormone concentration, so regulating TSH secretion (77). Other authors think this unlikely as it has been found that hormone concentration is higher in the adenohypophysis than in the hypothalamus in

several species (76) and that exogenous hormone is not more effective in suppressing thyroid function in hypothalamic lesioned animals.

From this literature it is quite possible to propose that there is a great possibility of the interaction of the endocrine pancreas and thyroid gland, which could be studied at the various levels of metabolism both in the thyroid gland and extrathyroidally. The available work on this interaction is not extensive and has been studied irrespective of approach to find the route of the effect of endocrine pancreas functional states particularly the hormone insulin on the thyroid gland activity.

Thus the objectives of this work are:

(a) To study the thyroid gland activity in rat under a diminished endocrine pancreatic functional state, i.e. alloxan diabetes and after pancreatectomy in rat and *in vitro* studies under the physiological conditions which pertain in alloxan and after pancreatectomy.

(b) To study the effect of exogenous administered insulin in rats on thyroid gland activity *in vivo*, also *in vitro* on the isolated thyroid gland of mouse.

(c) To study the effect of hypoglycaemic sulfonyl-ureas used in the treatment of diabetes mellitus, which enhance the internal insulin secretion, on the thyroid gland activity in rat and mouse.

(d) To study the effect of alloxan diabetes and insulin administration on thyrotrophin secretion in the rat and to examine the possibility of the existence of the role of thyrotrophin in the endocrine pancreatic-thyroid relationship.

GENERAL METHODS AND MATERIALS

Housing of animals

Female Wistar rats, T-O strain and Swiss-Webster Albino mice were used in the study. Almost all the *in vivo* and *in vitro* procedures involved the use of radio-iodide tracer techniques. The surgical technique of pancreatectomy was performed in some experimental procedures. All animals were maintained in a separate part of the animal house. The animals were housed in the unit for at least two weeks prior to being used for any experiment to acclimate to the environmental conditions. The surgical operations were performed in the surgical room of the animal house and in the post-operative period the animals were maintained in the separate unit.

Besides being a necessary safety measure, the separation of the experimental animals from the rest of the animal house had an advantage in that the conditions were quieter and only the author had access, thus unnecessary stress to the animals was avoided. It has been shown that noisy animal house conditions give rise to adrenal stimulation and increase in blood corticosterone levels (79) and corticosteroids have been shown to affect the thyroid (80) and extrathyroidal metabolism (81,82).

Room temperature was maintained at 21 $\pm 3^{\circ}$C and animals received a mixture of natural and artificial illumination from 7.30 a.m. to 7.30 p.m., humidity was not controlled. Stock rats were housed four per cage and received water and standard 41B diet *ad libitum*.

Radio-iodine administration

Radio-iodine as Na ^{131}I was bought from Amersham Radiochemical Centre, England. The maximum concentration of capacity was not more than 1 mci/ml in phosphate buffer with pH 7 to 8. The stock

solution was diluted as required with phosphate buffer of pH 8.0. In almost all experiments, radio-iodine was administered through an intraperitoneal route.

In vivo Measurement of radioactivity in the thyroid gland

Measurements of the activity of ^{131}I in the thyroid gland of the intact rats were taken, using the Brown-Grant technique (80) with modified "set-up".

The "set-up", Fig.1. consisted of a field scintillation counter, Type No.618A of EKCO Electronics, England, fixed vertically on the base. The proximal part of the thalium activated sodium iodide crystal of the counter was shielded with a cylindrical lead plate of 6.5 cm. in diameter and 4.5 cm in height. The lead shield completely covered part of the crystal and rested on the base of it. The proximal surface of the crystal was 2 cm. from the top of the shield exposed by an opening of diameter 2.5 cm.

A part of the lead shield was fixed in the anterior of a horizontal wooden platform of 36 cm. in length and 24 cm. in breadth, the top surface of the shield being level with the top surface of the platform. The surface of the platform was covered with a 0.25 cm thick transparent celluloid sheet. The horizontal platform was stabilised on four stands and clamps, which kept the apparatus undisturbed by the action of the rats upon the platform.

The scintillation counter was connected to an automatic scaler, type No.N610B of ECKO Electronics, England.

Unanaesthetized rats were used, unlike the conventional method which requires the use of diethyl-ether anaesthesia. It has been shown that diethyl-ether anaesthesia depresses thyroid activity in

rats. It depressed the uptake of ^{131}I and significantly inhibited the release of ^{131}I from the gland (83). For at least one week prior to being used for experimental counting, the rats were conditioned to handling without anaesthesia. As shown in Fig.2 animals were gently held from the dorsal surface of the neck and thorax and the thyroid gland region of the neck was faced against the proximal surface of the crystal of the counter on the horizontal wooden platform. The position of the gland against the crystal of the counter was corrected. While moving the neck region on the platform and counting the activity of the tracer on the automatic scaler, the position of maximum counts was considered to be correct. The thyroid region of the neck was counted for thirty seconds or for as long a time as possible to obtain the maximum counts for decreased statistical error (circa 10,000 counts).

Release of ^{131}I from the thyroid gland.

(A) In vivo

The technique of in vivo release of ^{131}I was performed in rats only, Fig.3. The rats were injected with the radioiodine tracer. 48 hours was allowed for the maximum uptake of the administered dose and for the maximum conversion of inorganic iodine into protein bound iodine or thyroid hormones (84). The total activity of ^{131}I in the thyroid gland was counted and considered as hundred per cent of the thyroid gland. The activity of ^{131}I in the thyroid gland was measured twice a day at ten and fourteen hour or nine and fifteen hour intervals for three to four days. These were considered as control period measurements. The animals were administered with the substance to be tested or the experimental conditions were induced. The measurement of the activity of ^{131}I

in the thyroid was carried on as required even after the treatment had been stopped. The correction of the decay of the radio-iodine tracer was calculated back to the time of the original measurement and considered as the percentage of the original measurement. The percentage of the activity of ^{131}I in the gland compared with the original measurement was plotted on a graph against the time from the first counting.

The slope of the line in the control period signifies the normal behaviour of release of ^{131}I from the thyroid gland. A decrease in the slope signifies an inhibition in the release of ^{131}I; an increase in the slope signifies stimulation of release of ^{131}I and no change in the slope indicates no effect on the release of ^{131}I from the thyroid gland, Fig. 3.

B. In vitro

The in vitro technique of release of ^{131}I was studied in the mouse only. The primary aim for the method of the study was to perform it under similar procedure as in the in vivo technique. An approach to evolve the technique was made by various steps. The various steps were modified until a practical shape for the technique had been evolved.

To study the viability of the isolated thyroid gland of the mouse, experiments of culturing thyroids in different culture solutions were performed. In one experiment, Krebs Ringer phosphate was added with the following substances; penicillin 50 U/ml., streptomycin 50µg/ml and neomycin 50 µg/ml and filtered through a millipore filter. The excised glands from the mice of T-O strain were incubated in 5 ml. conical flasks with 2 ml. of the buffer kept in a shaking incubator at a speed of 40 strokes/min. and temperature

regulated at 32 - 33°C. Different specimens of the gland were cultured for 48, 72 and 96 hours. At the end of the incubation period the glands were fixed in Bouin's fluid, prepared for wax embedding; 10 μ thick sections were cut and studied histologically. The glands were found to be unhealthy and altered after 48-hr. incubation, with increased severity after longer periods of incubation. In the 72-hr. incubated specimen, connective tissue had started to die, although the greater part of the follicular epithelium appeared histologically normal. In 96-hr. incubated specimens, reduction in colloid had occurred, the follicle had shrunk and epithelium had become taller in sequence.

After the failure to demonstrate the thyroid gland to be viable in the Krebs Ringer phosphate buffer, experiments in the "medium 199" were performed. 10 ml. of the medium 199 from Boroughs Wellcome & Co., London was added to 5 ml. of 4.4% $NaHCO_3$ and 5000 U penicillin; it was then made up to 100-ml. by normal dilution of the medium with deionised water. The excised glands of mice were cultured in a slightly modified watch glass by the method of Chen (85). To each dish 1.5 ml. of medium was added, and as shown in Figs.4 and 5, each dish was placed on a glass triangular support base and kept in a pair of petri-dishes. A triangular stainless steel sieved metallic grid was suspended in the medium in the dish and a piece of lens paper was placed in the centre of the upper surface of the metallic grid. The incubated thyroid gland was placed in the centre of the lens paper. Ten pairs of petri-dishes with ten incubated thyroids in the dishes were placed in a culture jar. The air of the jar was replaced by 95% O_2 and 5% CO_2. The jar was kept in a cooling incubator at a set temperature of 33°C. Different specimens of the gland were

incubated for 48, 72 and 96-hrs. and prepared for section cutting for study histologically after 48-hrs. and also after 72-hrs. incubation. In 96-hr. incubated specimens, the connective tissue seemed to have died to some extent but the follicular epithelium and colloid were found to be healthy and unaltered.

The experiments of the thyroid culture in medium 199 proved to be practicable for the further study of the thyroid gland *in vitro*.

The experiments were performed to establish a technique for the release of ^{131}I *in vitro*, similar to the study of *in vivo* release of ^{131}I from the thyroid gland by Brown-Grant (80), where the same gland is used for control and experimental periods. Mice of T-O strain were kept on low iodine diet for a week and each animal injected intra-peritoneally with 0.3 µci of radio-iodine in 0.1 ml. of buffer. Twenty-four hours after the injection of radio-iodine the animals were killed in groups of two or four with a blow on the head and the thyroid gland was excised from the animal in one piece and incubated in the prepared medium 199 by the watchglass method described in the previous paragraph. After every 4-hr. interval, the thyroids were transferred to a fresh lot of watch glasses with fresh medium, the previously used medium was collected in scintillation counting tubes and the activity of ^{131}I was counted in a well-type scintillation counter. This was continued for 36 - 48 hours after the start of incubation. The release of ^{131}I from the gland in the medium for the first four hours was considered as 100 per cent, while the release at other intervals was considered as the percentage release of the original four hours. Fig.6. shows the release of ^{131}I from the incubated thyroid gland of the mouse. Almost 99% of ^{131}I was released in the first four hours and was irregular throughout the regular periods of the incubation sampling.

This technique was carried out with further modifications in that the release of ^{131}I in the first eight hours was discarded and the release between the eighth and twelfth hours after the start of the incubation was considered 100 per cent and the release at other regular intervals as the percentage of it. Fig.7 shows the release of ^{131}I from the incubated thyroid gland of the mouse. Almost 60 per cent of the ^{131}I had been released between the fourth and eighth hours after the start of incubation. The release was not regular throughout the 36 hours of incubation.

The study of the release of ^{131}I *in vitro* on the same technique as *in vivo* was abandoned and procedure was further extensively modified. This proved quite practical.

In the further final modification, the incubated thyroid glands were divided into two groups of control and experimental. The medium of experimental thyroids had the substance to be tested added to it. The thyroid glands in the experimental and control dishes were incubated for the same time in the same experiment. This ranged from 12 - 24 hours in the different experiments. At the end of the incubation period the released ^{131}I in the medium and that left in the gland was measured. The released ^{131}I in the medium is considered as the percentage of total ^{131}I in the thyroid at the start of incubation or the total of the ^{131}I left in the thyroid and released in the incubation medium.

Uptake of ^{131}I and protein bound ^{131}I in the thyroid gland.

(A) *In vivo*

After the administration of radio-iodine, uptake of ^{131}I at a particular time was estimated by measuring the activity of ^{131}I at the thyroid region of the neck. The uptake was expressed against mg. wt. of thyroid gland, when the thyroid gland was excised out at the

end of the measurement of uptake, cleaned thoroughly from fatty tissue and weighed carefully.

In the study of the protein bound ^{131}I in the thyroid gland, the glands were homogenized and proteins of the gland were precipitated with the conventional trichloroacetic acid method. Precipitated proteins were centrifuged and washed with trichloroacetic acid (5%) once only. The activity of ^{131}I was measured in the precipitated proteins in a well-type scintillation counter and calculated as the percentage of total uptaken activity.

B. In vitro

The modified method of Singh and Chaikoff (86) was employed. The excised thyroid glands of mice were incubated from 4 to 8 hours in different experiments, in the normal culture medium. The glands were divided into two groups, experimental and control. The experimental glands were transferred to fresh watch glasses with fresh medium to which the substance to be tested was added, while the control glands were transferred to the watch glasses with fresh medium or the buffer if the substance to be tested for the experiment was dissolved in it. The glands were incubated for 4 to 16 hours and 0.2 μci of radio-iodine was added to each watch glass in 0.1 ml. of normal medium. The glands were incubated for a further six hours.

At the end of the incubation period, the activity of ^{131}I was measured in the gland, which gives the uptake of ^{131}I from the medium. Thyroids were weighed, homogenised and proteins of the glands were precipitated with trichloroacetic acid. The precipitated proteins were centrifuged and once washed with trichloroacetic acid. The activity of ^{131}I in the precipitated proteins was measured in a well-type scintillation counter and calculated as the percentage of the

total uptake of ^{131}I by the thyroid gland.

Plasma protein bound ^{131}I.

The measurements of plasma protein bound ^{131}I were performed by precipitating the blood plasma proteins using trichloroacetic acid and counting the activity of ^{131}I in the precipitated proteins. The animals were injected with radio-iodine at least 48 hours prior to the extraction of venous blood. The blood plasma was separated from cells by centrifugation at 1000 r.p.m. for an hour at $4^{o}C$.

Sterilisation of equipment and solutions.

Small glass apparatus was wrapped in aluminium foil. In the conical flasks and tubes tops were plugged with non-absorbent cotton wool, covered with aluminium foil and sterilised in an air-heated oven at $160^{o}C$ for at least one and a half hours.

Lens paper was cut to size for use in the culture and wrapped in aluminium foil. Lens paper, millipore filter unit and salt solutions were sterilised by autoclaving at 15 lbs./sq.in.pressure for 20 minutes.

Disposable, sterilised polythene test tubes were also used.

Extraction of blood samples.

Blood samples of rats for the estimation of blood sugar were taken from the tip of the tail region (not more than 0.2 ml. at a time). When killed, 5 ml. to 7 ml. of samples for the estimation of plasma protein bound ^{131}I were taken from the abdominal region of the inferior vena cava, extracted with a syringe, after opening the abdominal cavity of the rats.

Removal of thyroid gland.

Watchmakers' fine forceps and low power binocular microscopes were used for the dissection. After the removal of the thyroid gland from the trachea in rats, the gland was thoroughly cleaned of fatty tissue under the binocular microscope.

Determination of blood sugar and acetoacetate.

(a) Capillary blood sugar and acetoacetate.

"Blood sugar GOD-Perid-Method" with the prepared solutions from Boehringer Mannheim GMBH for blood sugar and the Salway (87) method of simultaneous determination of acetoacetate and glucose in blood capillary were employed.

(b) Urinary blood sugars.

For the qualitative determination of the urinary blood sugar in diabetic rats, the conventional Benedict's method was employed (78).

Drugs, other chemicals and diets used.

1. Alloxan monohydrate — Sigma Chemical Co.Ltd.
2. Insulin (Crystalline) from bovine pancreas — Sigma Chemical Co.Ltd.
3. Diketene (Acetoacetic anhydride) — Sigma Chemical Co.Ltd.
4. Thytropar® Thyroid stimulating hormone — Armour Pharmaceutical Co.Ltd.
5. Medium 199 for tissue cultures — Boroughs Wellcome Laboratories
6. Sodium bicarbonate, sterile, 4.4% aqueous solution — Boroughs Wellcome Laboratories
7. Penicillin G. Crystalline, as sodium salt — Sigma Chemical Co.Ltd.
8. Streptomycin sulphate B.P. — Glaxo Laboratories Ltd.
9. Neomycin sulphate B.P. — Glaxo Laboratories Ltd.
10. Analar grade Salts — Hopkins and Williams Ltd.
11. D-glucose S.L.R.(Anhydrous) — Fisons Scientific Apparatus Ltd.
12. Blood sugar GOD-perid®-Method kit — Boehringer Mannheim GMBH
13. Rastinon, tolbutamide as sodium salt — Hoeschst Pharmaceuticals Ltd.
14. Glibenclamide — Hoeschst Pharmaceuticals Ltd.

15. Halothane B.P. Imperial Chemical Industries Ltd.

16. "Low iodine" test diet (formulated per Remington diet No.347) Nutritional Biochemical Co., Cleveland, Ohio, U.S.A.

17. 41B diet

18. Bovine serum albumin (Fraction v, powder) Sigma Chemical Co.Ltd.

Expression of results and statistics

Thyroidal iodide (^{131}I) uptake was expressed as mg.^{wet} weight of the gland *in vivo* and *in vitro* and the weight of the gland as a percentage of body weight in *in vivo* studies. In all *in vitro* and some of the *in vivo* studies the protein bound thyroidal iodide (^{131}I) was expressed as the percentage of total iodide uptake (^{131}I) by the gland. TSH levels were expressed as in the percentage of blood volume.

In group data the mean, standard error and t values were calculated by computer. Comparisons between control and experimental groups as well as between groups were made and a statistically significant difference between groups was taken as $P = 0.05$ or $P<0.05$.

Fig.1 & 2. The "set up" for *in vivo* counting of radioiodine from the thyroid region of the neck of intact animals.

Fig.3. The technique for the rate of the release of radioiodine from thyroid gland of intact animals.

A - Inhibition of release

B - No effect

C - Stimulation of release

Fig.4 & 5. The "set up" for *in vitro* studies on the thyroid gland.

Fig.6 & 7. *in vitro* Release of radioiodine from the thyroid gland of the mouse labelled *in vivo*.

Fig.1.

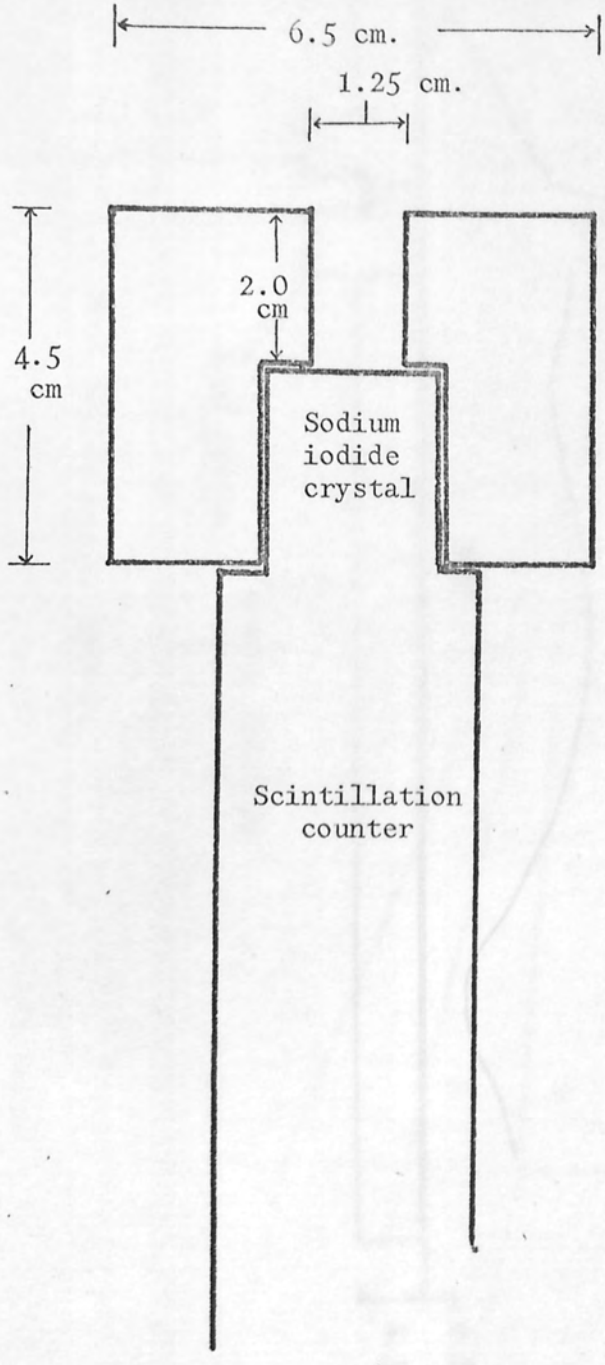

6.5 cm.
1.25 cm.
2.0 cm
4.5 cm
Sodium iodide crystal
Scintillation counter

Fig.2.

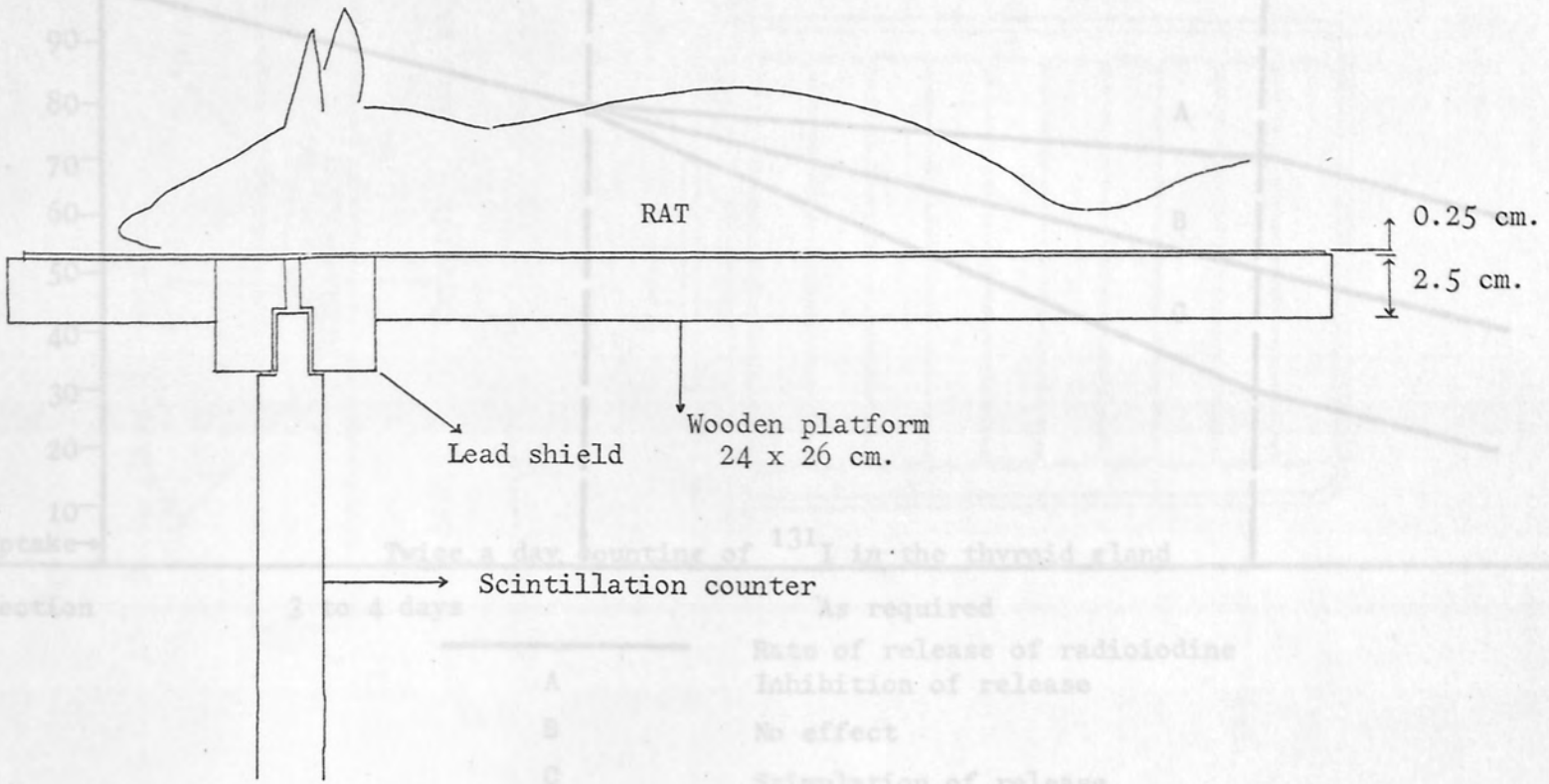

RAT
0.25 cm.
2.5 cm.
Lead shield
Wooden platform
24 x 26 cm.
Scintillation counter

Fig.3.

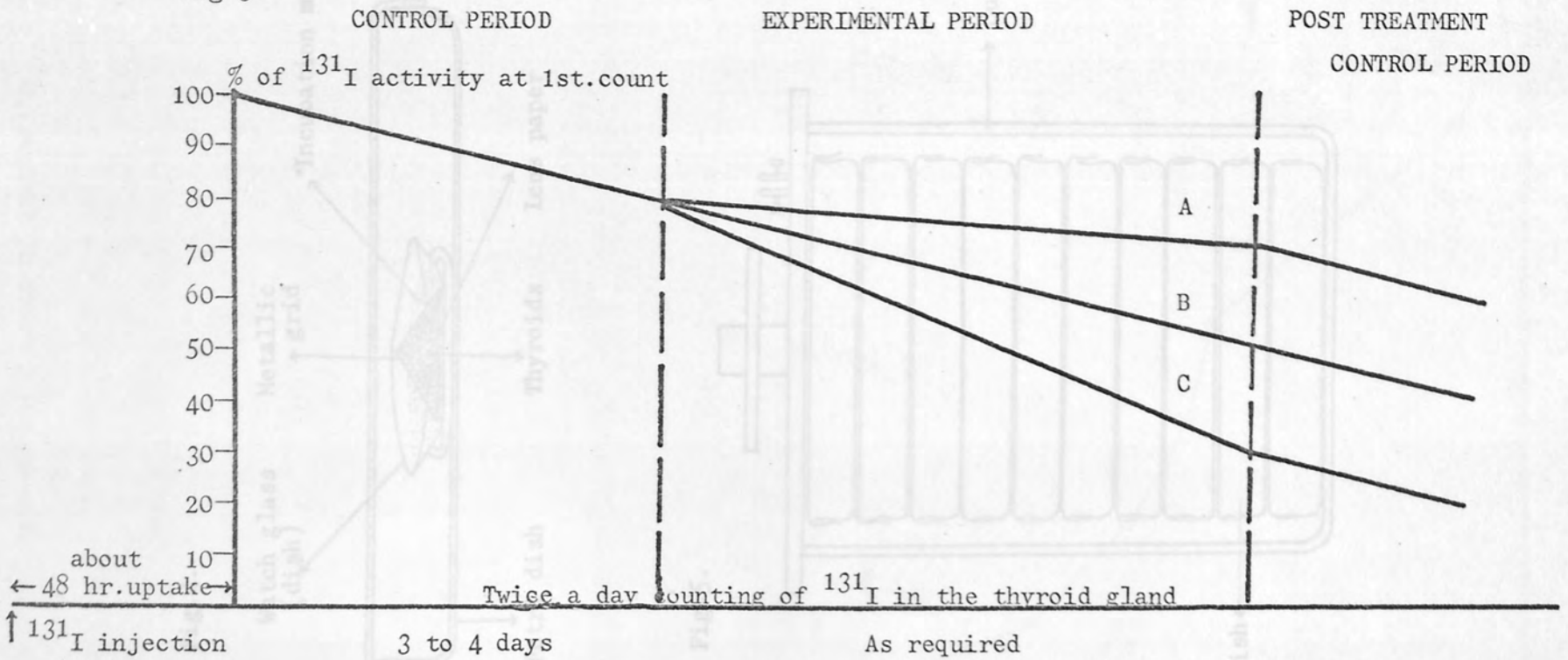

CONTROL PERIOD
EXPERIMENTAL PERIOD
POST TREATMENT CONTROL PERIOD
% of 131I activity at 1st.count
100
90
80
70
60
50
40
30
20
about 10
←48 hr.uptake→
A
B
C
Twice a day counting of 131I in the thyroid gland
131I injection
3 to 4 days
As required
A Rate of release of radioiodine Inhibition of release
B No effect
C Stimulation of release

Fig.4.

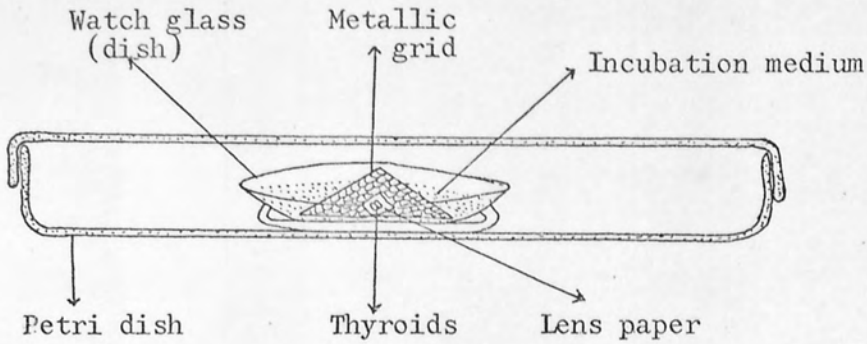
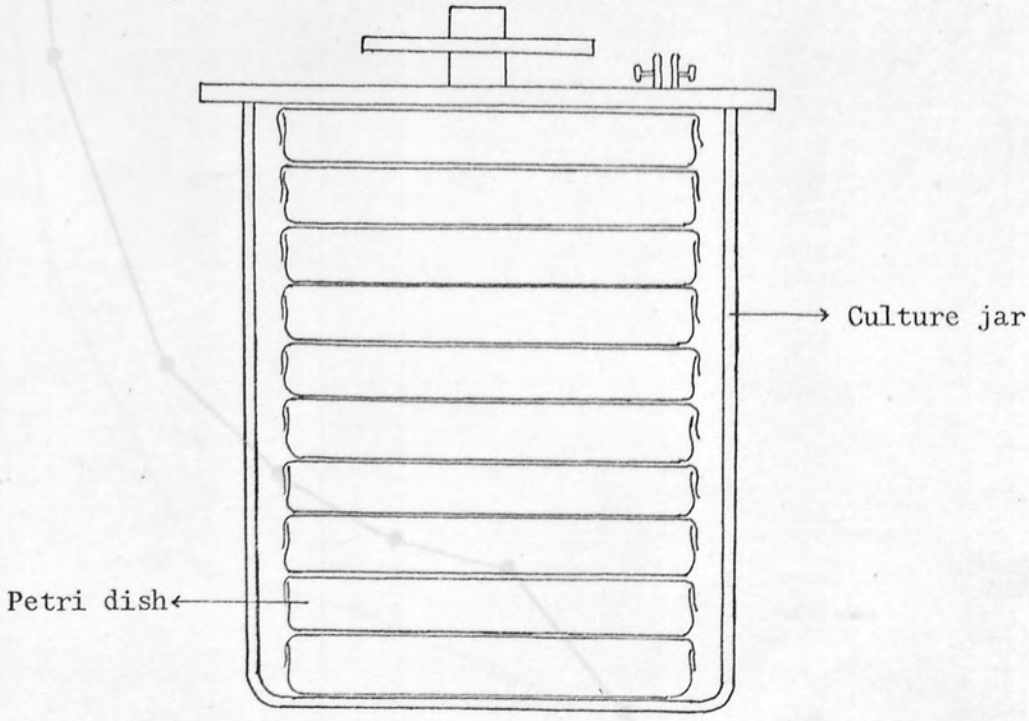

Watch glass
(dish)
Metallic
grid
Incubation medium
Petri dish
Thyroids
Lens paper

Fig.5.


Culture jar
Petri dish

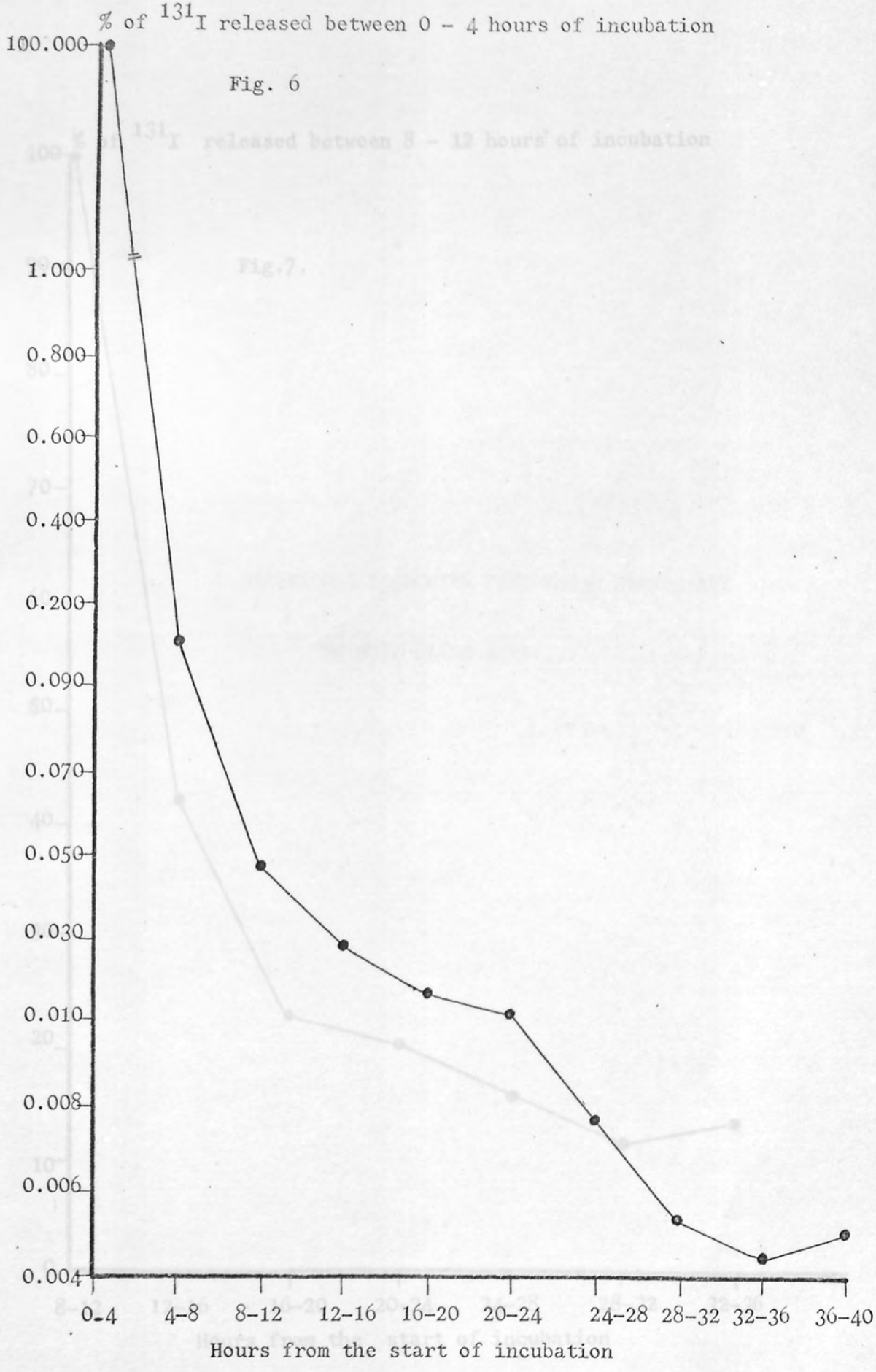

% of ^{131}I released between 0 - 4 hours of incubation
100.000
1.000
0.800
0.600
0.400
0.200
0.090
0.070
0.050
0.030
0.010
0.008
0.006
0.004
0-4
4-8
8-12
12-16
16-20
20-24
24-28
28-32
32-36
36-40
Hours from the start of incubation

Fig. 6

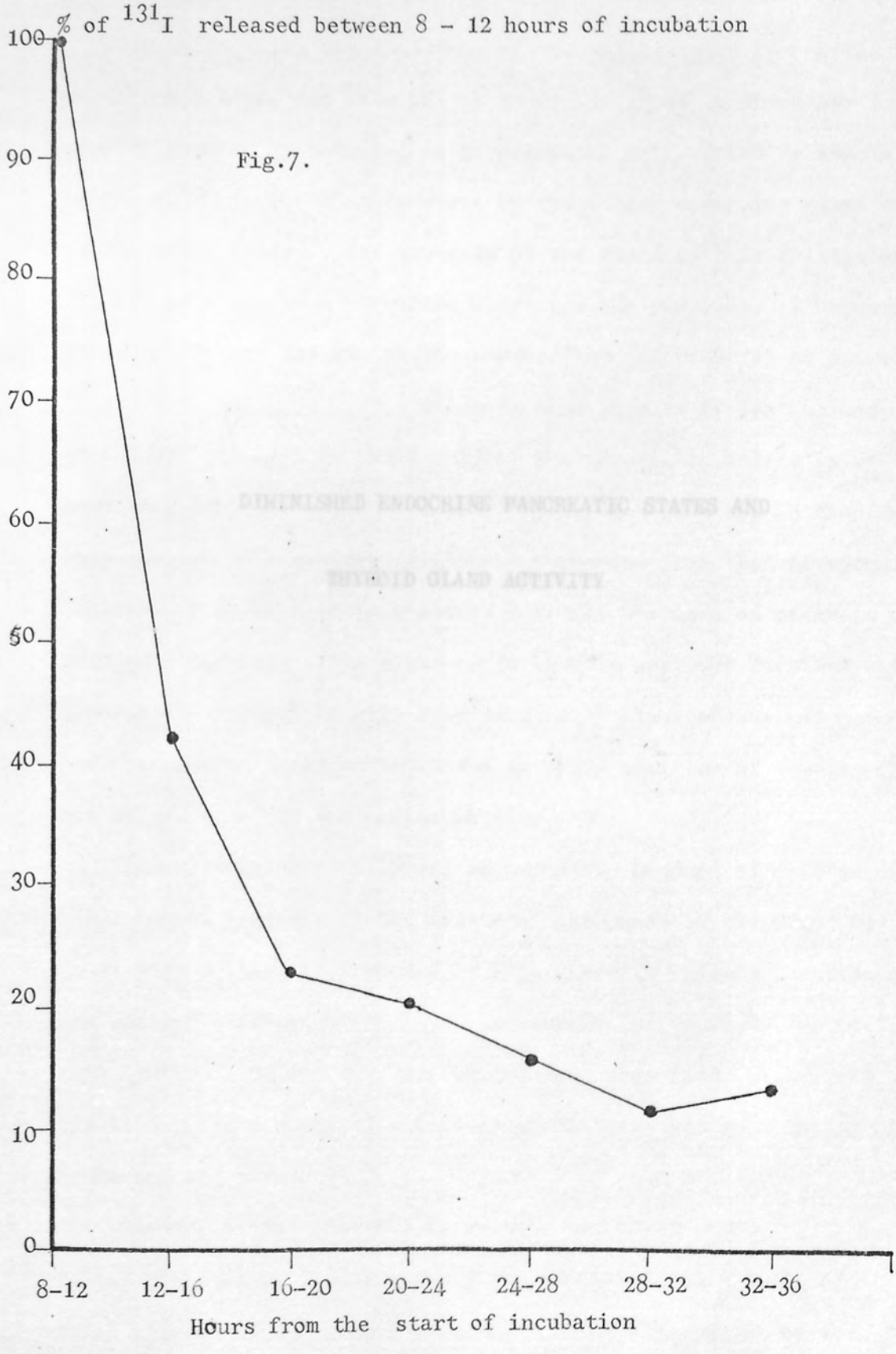

% of ^{131}I released between 8 - 12 hours of incubation
100
90
80
70
60
50
40
30
20
10
0
8-12
12-16
16-20
20-24
24-28
28-32
32-36
Hours from the start of incubation

Fig.7.

DIMINISHED ENDOCRINE PANCREATIC STATES AND THYROID GLAND ACTIVITY

INTRODUCTION

In maintaining the constancy of the milieu interieur of the endocrine system, the role of one endocrine gland to the other is usually studied by inducing an experimental malfunction or removal of the gland. The changes shown by the target endocrine gland are considerable evidence for the role of the gland in this relationship.

Insulin has been extracted only from the pancreas, it appears that significant amounts of the hormone are not produced or secreted by other tissue (88). The evidence that insulin is synthesised, stored and secreted by the β-cell of the pancreatic islets is of such long standing as to need no elaboration. The application to this problem of a somewhat different technique from that previously employed is perhaps worth comment. Frozen sections of pancreas from many species, after exposure to insulin antibody labelled with fluorescein isocyanate have been studied. Fluorescence was noted and fluorescent reaction inhibited by prior addition of insulin (but not of glucagon) to the antiserum (89).

Administration of alloxan, an oxidative product of uric acid (90), causes necrosis of the islets of the pancreas (91,92,93,94). Islet degeneration is preceded by hyperglycemia (95,96). Alloxan has induced a permanent diabetes in rabbits (94,97,98,99,100,101), rats,(102,103,104,105,106,107,108,109) and dogs (110,111,112,113, 114,115). In successful experiments, diabetes was apparent in 24 hours and was permanent.

Removal of the pancreas is another method of producing experimental diabetes by inducing the state of diminished insulin or total absence of endogenous insulin. Total extirpation of the

pancreas in the dog and the cat results in the immediate onset of severe diabetes and marked impairment of the intestinal absorption of food (116,117,118). Severe diabetes could not be produced after total pancreatectomy in the rat (119,120,121,122,123,124,125), while other authors have shown that within a day after the operation rats develop severe diabetic symptoms; if not treated with insulin they die within 48 hours in a diabetic coma (126,127).

The insulin insufficiency, due to the necrosis of beta cells, is directly or indirectly associated with abnormal metabolism of proteins and fats which leads to the crisis of the disease. The diabetic animals may show impaired fat metabolism by a high rate of formation of the ketone bodies. It has been shown that various fatty acids are oxidised to ketone bodies in the liver (128,129,130, 131,132).

Early studies involving insulin and fatty acid metabolism led to the suggestion that complete oxidation of fatty acids without ketone formation could not be carried out unless an adequate supply of carbohydrate was present. This was expressed by the statement that "fat burns in the flame of carbohydrate". Later when the nature of the biochemical mechanisms of metabolism were more clearly and completely outlined, the relationship between fatty acid metabolism and carbohydrate oxidation and ketone formation was more clearly defined and the suggestion was made that impaired carbohydrate metabolism in the diabetic animal led to a catabolism of fatty acids. Such a shift was seen to result in an excess formation of 2-carbon acetate fragments. Thus the tissues appeared to be flooded with more substrate than could be utilized and the excess acetate molecules condensed to form the acidic "ketone bodies", acetone, acetoacetic acid and betahydroxybutyric acid; an abnormally high level of these

compounds in the blood constitutes "hyperketonemia" (133). Insulin exerts a direct modification on the fatty acid metabolism to account for the ketone formation, as it has been reported that the ketonemia regularly following the injection of anterior pituitary extract was suppressed in rabbits by the simultaneous administration of insulin (134). This effect has been attributed to the specific action of insulin upon the liver in preventing hepatic glycogenolysis.

The work described here is an attempt to study the aspects of the thyroid gland activity under the conditions of diminished insulin in the pancreas, induced by alloxan administration and after pancreatectomy in the rat. Also to examine the effect of administration of the substances produced in diabetes, i.e. glucose and acetoacetate in the rat and their effect on the isolated thyroid gland of the mouse in vitro.

METHODS AND MATERIALS

Alloxan diabetes: The experiments in this category consisted of two groups, one to show the spontaneous effect and the other the effect of prolonged diabetes induced by alloxan.

(a) Spontaneous effect after diabetes

Twelve rats weighing 200 - 230 gms. were selected and numbered 1 - 12. Each rat was injected with 10 μci of radio-iodine intraperitoneally. The rate of the release of ^{131}I from the thyroid gland was determined. The activity of ^{131}I of the neck was counted for six times in the control period. Immediately after the seventh time of counting, 150 mg/kg of body weight of alloxan monohydrate dissolved as 20 mg/ml (or molarity of 0.14) in McIlwaine's citrate phosphate buffer at pH 4.0 (102) was injected to each rat.

Rats No.6 and 7 died within fifteen to thirty hours after the injection of alloxan monohydrate. After thirty hours, the urinary reducing sugars were estimated. All the rats except Nos.2,8 and 12 showed considerable diabetic conditions. These three rats were further injected with 100 mg/kg. of body weight of alloxan. Five rats, Nos.2,8,9,10 and 11 died during the experiment. The remaining five rats survived throughout the experiment. The neck region of the thyroid gland was counted for fourteen times after the control period. The background and decay were corrected.

The blood samples for the estimation of blood sugar and acetoacetate were taken just before the fifth and twelfth counting of the neck region.

(b) Prolonged diabetes

Eighteen, of twenty-four rats weighing between 230 and 250 gms. were treated with 150 mg/kg. of alloxan. Five rats died of alloxan shock in the next four days. The blood sugar level of the rest was estimated. The level of most of the animals ranged between 380 - 593 mg% except five, who ranged from 133 - 193 mg%. The animals with low blood sugar level were further injected with 50 mg/kg. of body weight of alloxan. The blood sugar level of these animals was estimated, three developed considerable hyperglycemia with the blood sugar level ranging between 393 - 453 mg%, the next two were mildly diabetic with levels ranging 126 - 152 mg%. These mild diabetics were not further treated with alloxan, but kept as a separate group of mild diabetics. All the animals were kept for a further twenty days. Three of the remaining eleven severe diabetics died within this time. Thus, two mild diabetics and

eight surviving severe diabetics were in such a state for the total of 28 days. The untreated six animals were kept as controls under similar housing conditions.

28 days after the first injection of alloxan, 10 µci of ^{131}I ($Na^{131}I$) was injected to each rat intraperitoneally. Uptake of ^{131}I by the thyroid gland was estimated at 4, 24, and 45 hours after the administration of radio-iodine. 46 hours after the radio-iodine administration, the animals were killed by a blow on their heads and 5 - 7 ml. of venous blood was taken out to estimate the plasma protein bound ^{131}I. The thyroid gland was excised out, cleaned off from fatty tissue and carefully weighed on a sensitive balance.

Pancreatectomy.

The experiments in this category consisted of two groups: Total pancreatectomy and partial pancreatectomy.

(a) Total pancreatectomy

Almost two dozen rats weighing 170 - 190 gms were surgically operated for total pancreatectomy by a procedure based on the method of Scow (127). About a dozen of these rats were utilized to master the technique, as total pancreatectomy in the rat is quite difficult because of certain peculiarities of the pancreas in this species. After a fast of 12 - 18 hours, the animals were operated under a continuous supply of anaesthesia halothane, nitrous oxide and oxygen. The removal of the pancreas was completed in three steps, with the aid of a binocular microscope (10x magnification).

A mid-line abdominal incision was made. The stomach and spleen were pulled out of the abdominal cavity and placed on the chest wall. The transverse colon and its mesocolon were gently pulled cauded to expose the origin of the splenic vessels. With fine-

tipped forceps the pancreatic tissue overlying the junction of the portal and splenic veins and along the first few millimetres of the splenic vessels was gently lifted cephalad without separating it from the rest of the gland. With the rat so that its head pointed to seven o'clock, the spleen was taken between the left thumb and index finger with the finger tip placed under the splenic vessels. Starting in the abdomen and working towards the spleen with swabs (cotton wool rolled on tips of round tooth picks), the pancreas was pushed cephalad off the splenic vessels and off the vessels in the hilum of spleen. The gastrosplenic ligament was separated from the spleen and was lifted on to the stomach. Any fragment of pancreatic tissue separated from the larger mass was immediately removed with fine-tipped forceps. The spleen was then returned to the abdominal cavity. The rat was rotated so that the head pointed to twelve o'clock and the gastrosplenic ligament was taken between the left index finger and thumb. Starting at the cardiac end, the gastrosplenic ligament was separated from the stomach. When pancreatic tissue was encountered it was pushed away from the stomach and blood vessels towards the tissue mass in the left hand. When a band of pancreatic tissue was left across the front of the pylorus, the ligament was pulled out and the band was placed on the left index finger. The tissue on this finger was rubbed away from the abdomen with swabs to separate pancreatic tissue from the enclosed, large pancreatic duct. After exposing the duct, it was cut with scissors. Later, the remaining segment of this duct was cut with the electrocautery close to the bile duct.

The duodenum was pulled out of the abdomen and to the right side of the rat. The colon was gently pulled to the opposite side

in order to separate the mesoduodenum and mesocolon so as to expose the portal vein. The rat was now rotated so that the head pointed to two o'clock. The duodenum, taken between left thumb and index finger was pulled to the right side of the rat and the finger tip was placed under the bile duct. With cotton wool swabs the pancreas was pushed away from the bile duct towards the abdomen, starting at the duodenum and working towards the liver. About 0.2 cm. from the duodenum the large pancreatic duct from the second portion of the pancreas was found and was cut near the bile duct with electrocautery As the little lobules of the pancreas in the bile duct were isolated their ducts were cut either with the electrocautery or by pulling them apart between two pairs of forceps. About halfway between the duodenum and the liver the pancreas is found entering the bile duct. At this time the pancreatic tissue which lies along the course of the gastro-duodenal vessels was separated from these vessels, the duodenum and pylorus. The above pancreatic duct lies within this small mass of tissue. The dissection was continued along the blood vessels until the bile duct was encountered, at which time the pancreatic duct was cut next to the bile duct with the electrocautery. The animal was rotated so that the head pointed to eight o'clock. The duodenum was taken with the left index finger and thumb and pulled to the left side of the animal, the finger tip was placed under the bile duct. The remaining pancreatic tissue between the bile duct and the duodenum, as well as along the bile duct, was then removed. Near the liver the pancreas was difficult to see if the bile duct was not pulled out a little. The bile duct was cleaned as much as possible.

After rotating the rat so that the head pointed to nine o'clock, the duodenum, taken between the left thumb and index finger was pulled to the left side of the rat and the finger tip was placed under the inferior pancreaticoduodenal vessels. The peripheral edge of the pancreatic tissue along the back of the abdomen was first lifted with fine-tipped forceps and then with swabs. This pancreatic tissue and that along the inferior pancreatic duodenal vessels was pushed toward the duodenum. After cleaning halfway to the duodenum, the other half of these vessels was cleaned by working from the opposite direction. The pancreatic tissue freed from these vessels was lifted cauded. Starting at the junction of these vessels with the duodenum, the pancreatic tissue lying along the duodenum was carefully pushed toward the mesentery and then freed. The duodenum was then pulled to the right side of the rat and the portal vein was carefully inspected for pancreatic segments, especially near its junction with duodenal and splenic vessels. The abdominal wall and the skin were closed with a continuous cotton suture.

(b) Partial pancreatectomy

Twelve, of the eighteen rats weighing between 200 and 230 gms. were partially pancreatectomised. The portion, consisting of the pancreatic tissue which lies in the gastrosplenic ligament and along the splenic and gastroepiploic vessels and the portion in the mesoduodenum posterior to the bile duct were removed. The portion consisting of small lobules along the full length of the bile duct was left intact. Also the diffused pancreas between the blood vessels was not swabbed completely where it could have led to severe haemorrhage. Six unoperated rats were kept as controls.

Two rats died within 48 hours, probably due to surgical trauma.

After five weeks from the surgical operation the blood sugar and acetoacetate levels were estimated in both operated and control animals.

After eight weeks from the day of surgical operation, 5 µci of ^{131}I (Na^{131}) was injected intraperitoneally to each animal and uptake of ^{131}I was determined at the first, fourth and eighth hours after the administration of radio-iodine. 48 hours after the administration of radio-iodine, the animals were killed by a blow on the head region and the thyroid gland was excised out, cleaned free of fatty tissue and weighed carefully on a sensitive balance. 5 - 7 ml. of venous blood was taken out to estimate the plasma protein bound ^{131}I.

Acetoacetic anhydride (Diketene) administration.

Fourteen rats weighing between 200 - 230 gms. were used. Diketene was prepared in phosphate buffer of pH 8.0. Eight rats were administered with 4 mg/kg. of diketene twice a day for twenty-eight days. The injections were carried out at 10.00 and 20.00 hours every day. The remaining six were injected with phosphate buffer of pH 8.0 only for the same time and considered as controls.

Fourteen hours after the last injection, 10 µci of ^{131}I ($Na^{131}I$) was administered intraperitoneally to each rat of the experimental and control group. Uptake of ^{131}I was determined at 8, 23 and 72 hours counting, for the determination of the uptake of ^{131}I. Seventy-four hours after the first injection of the radio-iodine the animals were killed with a blow on the head. The thyroid gland was removed, cleaned of the fatty tissue and carefully weighed on a

sensitive balance. 5 - 7 ml. of venous blood was taken and plasma protein bound ^{131}I was determined.

In vitro glucose.

Isolated thyroid glands of T-O strain mice were incubated in varying concentrations of the glucose in the incubation medium. Ten, seven, six, five and five paired thyroids were incubated in zero, 1, 2, 4 and 8 mg/ml. of glucose in the medium. The medium without glucose was considered as the control thyroid gland. The control thyroids were distributed in groups of two or three in the culture jar among the experimental thyroids.

After sixteen hours of incubation, 0.2 µci of ^{131}I ($Na^{131}I$) was added to each dish. The thyroids were incubated for a further six hours. At the end of the incubation period each gland was put in a separate test tube and the total activity of ^{131}I uptaken was determined. These were carefully weighed on a sensitive balance, homogenized and treated with trichloroacetic acid for the determination of protein bound ^{131}I in the gland.

In vitro diketene.

Isolated thyroid gland of T-O strain mice was incubated with varying concentrations of diketene in the incubation medium. Two experiments were performed, one with high doses, the other with pharmacological/physiological doses after the high doses showed the chronic effect on the thyroid gland activity.

(a) High doses

Ten for the zero and five thyroids each for the 0.2, 1.0, 2.0 and 3.0 mM concentrations of diketene in the medium were incubated. The medium without diketene was considered as control.

After twelve hours of incubation 1.8 μci of ^{131}I ($Na^{131}I$) was added to each dish. Thyroids were incubated for a further six hours. Each gland was put in a separate test tube and total ^{131}I uptaken was determined. The thyroids were carefully weighed in a sensitive balance, homogenised and treated with trichloroacetic acid for the estimation of protein bound ^{131}I in the gland.

(b) Pharmacological/Physiological doses

Fifteen, five, ten, ten, five and five paired thyroids were incubated in zero, 0.2, 0.4, 0.6, 0.8 and 1.0 mM concentrations of diketene respectively in the incubation medium. Further procedures were performed as described in the experiment with high doses.

RESULTS

Alloxan diabetes

(a) Spontaneous effect after diabetes

The results of this experiment, representing an average of the five of the twelve animals which survived at the end of the experiment are shown in Fig.8. A regular release of ^{131}I from the thyroid gland was shown in the control period. The rate of the release of ^{131}I remained unaltered from the control period, to three days after the treatment of alloxan. It was significantly inhibited afterwards. The inhibition of the release rate of ^{131}I remained regular and did not show any reversible behaviour similar to the release rate during the control period until the end of the experiment.

The results of the blood sugar level estimations and the aceto-acetate concentration of the samples are shown in Fig.9. The average blood sugar level just after the fifth counting, i.e. 24 hours before the alloxan treatment was 86 ±8 mg%. It increased to 500 ±32 mg% just after the twelfth counting, i.e. 85 hours after the

treatment with alloxan. The average blood acetoacetate level just after the fifth counting was .1±.001mM and it increased to 1.813 ±0.058 mM just after the twelfth counting.

(b) Prolonged diabetes

Fig.10 shows the uptake of ^{131}I calculated as mg/wt. of the thyroid gland. The uptake of ^{131}I by the thyroid gland of the diabetic animals was significantly lower than the normal animals; measured at 4, 24 and 45 hours after the administration of radio-iodine. The thyroid gland of the mild diabetics failed to show any difference in the uptake of ^{131}I when compared with the normal rats.

In Fig.11 the average weights of thyroids per hundred gms. of body weight are shown. The weight of the thyroid gland of the severe diabetics had significantly increased over that of the normal animals ($P > 0.01$). The mild diabetics failed to show any significant difference.

In Fig.12 the average of the plasma protein bound ^{131}I ($PB^{131}I$) levels is shown. The levels are expressed as the counts of the activity of ^{131}I in the precipitated proteins per 1000 seconds. The level in the severely diabetic rats had been significantly lowered ($P > 0.001$). Also the level in the mild diabetics was significantly lowered ($P>0.05$).

Pancreatectomy

(a) Total pancreatectomy

The animals with complete pancreatectomy could not be successfully used for the performance of the experiments on thyroid gland activity. In addition to weakness, hypothermia, prostration and hyperventilation, the rats developed diabetic coma and had markedly

swollen abdomens. Almost all operated animals died within 48 hours after the pancreatectomy.

This approach for monitoring the performance of the thyroid was abandoned because it was felt that the thyroid gland activity in the total pancreatectomized rat could not be attributed only to the insufficiency of pancreatic tissue, for a lot of other factors originating from the surgery could be responsible for any alteration seen in the activity of the thyroid gland.

(b) Partial pancreatectomy

The results of the blood sugar level and acetoacetate of the samples taken from the partial pancreatectomised and the unoperated rats are shown in Fig.13. The average blood sugar levels of unoperated and the partial pancreatectomised were 62 $\pm$2 mg% and 96 $\pm$6 mg% respectively. The average blood acetoacetate levels of unoperated and partial pancreatectomised were 0.103 $\pm$0.009 mM and 0.101 $\pm$0.008 mM respectively. The blood sugar level in the partial pancreatectomised animals was significantly higher than in the unoperated, but the level was not high enough to describe the animals as diabetics.

Fig.14 shows the uptake of ^{131}I calculated as mg/wt. of thyroid gland. The uptake of ^{131}I by the thyroid gland of the partial pancreatectomised animals was significantly lower than the unoperated animals; measured at 1 and 8 hours after the administration of the radio-iodine. However, it failed to show significant difference at 4 hours uptake.

In Fig.15 the average weights of thyroids per hundred gms. of body weight are shown. The weight of the thyroid gland of partial pancreatectomised rats failed to show any difference from that of the unoperated rats.

In Fig.16 the average of the plasma protein bound ^{131}I($PB^{131}I$) is shown. The levels are expressed as the counts of the activity of ^{131}I in the precipitated proteins for 1000 seconds. The level in the partial pancreatectomized rats was significantly lower than the unoperated animals ($P > 0.001$).

Acetoacetic anhydride (diketene) administration

Fig. 17 shows the uptake of ^{131}I calculated as mg/wt. of the thyroid gland. The uptake of ^{131}I by the thyroid gland of diketene administered animals was significantly lower than the control animals; measured at 8, 24, and 72 hours. However, the difference in the uptake of ^{131}I between diketene administered and control animals decreased with the increased gap of the time from the withdrawal of diketene administration.

In Fig.18 the average weights of thyroids per hundred gms. of body weight are shown. The weight of the thyroid gland of diketene administered rats failed to show any difference from that of the control rats.

In Fig.19 the average of the plasma protein bound ^{131}I ($PB^{131}I$) is shown. The levels of the ^{131}I in the precipitated proteins was significantly lower in the diketene treated rats than in the normal rats ($P > 0.001$).

In vitro glucose.

Fig.20 shows the uptake of ^{131}I expressed as counts/mg. wet weight of thyroid gland of the mouse. The uptake of ^{131}I is expressed against the different concentrations of the glucose in the incubation medium. Glucose concentrations as high as 8 mg/ml. or 800 mg% of incubation medium failed to show any effect on the uptake

of ^{131}I by the thyroid gland when compared with the thyroids incubated in the medium without any glucose.

In Fig.21. the percentage of the protein bound activity of ^{131}I in the thyroid gland of the mouse is shown. The protein binding is expressed against the different concentrations of glucose in the incubation medium. Concentrations as high as 8 mg/ml.(800 mg%) failed to show any effect on the protein binding of ^{131}I when compared with the thyroids incubated in the medium without any glucose.

In vitro diketene.

High doses

Fig.22 shows the uptake of ^{131}I expressed as counts/mg. wet wt. of thyroid gland of the mouse. The uptake is expressed against the different concentrations of the glucose in the incubation medium. The uptake of ^{131}I was significantly higher in the thyroids with 0.2 mM of diketene, while the other concentrations of 1.0, 2.0 and 3.0 mM of diketene in incubation medium showed a gradual decrease in the uptake with the increased concentration of diketene in the incubation medium.

In Fig.23, the percentage of the protein bound activity of ^{131}I in the thyroid gland of the mouse is shown. The protein binding is expressed against the different concentrations of glucose in the incubation medium. The concentration of 0.2 mM of diketene failed to show any effect while 1.0, 2.0 and 3.0 mM concentrations in the incubation medium showed a gradual decrease in the protein bound ^{131}I with the increased concentration of diketene.

Pharmacological/physiological doses

Fig.24 shows the uptake of ^{131}I expressed as counts/mg.wet wt. of thyroid gland of the mouse. The uptake is expressed against the

different concentrations of the diketene in the incubation medium. With the exception of 0.2 and 0.8 mM of diketene, there was a gradual decrease in the uptake of ^{131}I with the increased concentration of diketene in the incubation medium.

In Fig.25, the percentage of the protein bound activity of ^{131}I in the thyroid gland is shown. The protein bound activity is expressed against the different concentrations of diketene in the incubation medium. The concentration of 0.2 mM of diketene failed to show any effect, otherwise there was a gradual decrease in the protein binding of ^{131}I with increased concentration of diketene in the incubation medium.

Table 1 shows the uptake of the ^{131}I in the control and the experimental; and the plasma protein bound ^{131}I of the control and the experimental animals. In all the experiments with alloxan diabetic, partial pancreatectomized and acetoacetic administered rats, the ratio of protein binding was lower than the ratio of uptake.

DISCUSSION

All the experiments in this work have been shown to evoke an antithyroid state. These states are in three groups: a state of diminished insulin along with the production of hyperketonemia, induced by alloxan administration; a state of diminished insulin without hyperketonemia induced by partial pancreatectomy and a state of presence of products of hyperketonemia and hyperglycemia in the in vitro studies. All the experimental states show that both the diminishing of insulin and production of "ketone bodies" are responsible for the antithyroid activity in the diabetic conditions.

The antithyroid activity has been shown at all the levels by the uptake of iodide or the transport of the iodide through the

membrane of the thyroid gland by the coupling of the iodine with the protein in the thyroid gland for the production of thyroid hormone and the proteolysis by the release of the hormone from the thyroid gland.

It has been shown that the presence of oxygen is necessary for iodide transport as the inhibitors of the oxidative process depress the T/M(I^-) more or less in parallel with oxygen consumption (135,136,137,138,139). Oxidative phosphorylation must be intact for iodide transport to occur, various agents that uncouple oxidative phosphorylation such as 2,4-dimitrophenol depresses T/M both for iodide and for certain other anions (137,139,140). The ability of the thyroid to concentrate iodide is markedly diminished at low temperatures (136,139). The pH optimum for I^- concentration in the thyroid slices is 7.5 to 7.8, the deviation from this optimum affected the T/M ratio for iodide (141). Deletion of Na^+ and K^+ from Ringer medium in which the thyroid gland slices are incubated or leaching iodide concentrating tissue with K^+ free Ringer medium lowered the T/M(I^-) (142,143). Certain specific transport inhibitors of ion transport acting probably via an inhibition of the Na^+__,K^+__ require ATPase activity, which controls K^+ and Na^+ transport and loss of which in turn depresses iodine transport (142,143,144,145,146).

The alloxan diabetic rats in this work had shown polyuria, the consumption of the water was six times that of the normal rats; this polyuria followed by polydipsia leads to hypothermia. It has already been shown that hypothermia occurred in the total pancreatectomised-diabetic rats (127). The decrease in the body temperature could be attributed to the decreased uptake of iodine which seems unlikely, because the decrease in the uptake of iodine was observed

even in the diketene administered rats without the symptoms of polyuria.

Hyperglycemia in the diabetics could not be responsible for the antithyroid activity as it failed to show any adverse effect in the uptake and protein binding of iodine in the thyroid gland. This leaves the ketone bodies as the possible factors inducing hypothyroid conditions.

The primary role of 'ketone bodies' in the interference of transport of iodide could be clearly evalued from the results in the partial pancreatectomised rats. Hyperketonemia was not observed in these rats and these rats failed to show any dramatic difference in the uptake of iodine by the thyroids compared with normal rats as was shown by the alloxan diabetic and acetoacetic administered rats.

For each molecule of betahydroxybutyrate and acetoacetate formed, a hydrogen ion is also formed and so a severe hyperketonemia causes a fall in the blood pH; this causes ketoacidosis (87). The fall of blood pH in the ketoacidosis could be attributed to the influence in the transport of I^- in the alloxan diabetics but in the light of the experiments of acetoacetic administration it cannot be considered to be the only factor responsible as significant decrease in the uptake of radio-iodine occurred in the acetoacetic administered rats in the absence of ketoacidosis.

Depletion of Na^+ and K^+ with the polyuria and polydipsia could be a factor in the interference of the iodide transport, but the possibility is in question in the light of the experiments with acetoacetic administered rats, where the interference with the iodide transport was observed in the absence of polyuria and polydipsia.

The products of hyperketonemia might be affecting the iodide transport by acting as inhibitors of the oxidative process or uncoupling oxidative phosphorylation. These may be acting as specific transport inhibitors which act via an inhibition of the $Na^{\pm}$ and $K^{\pm}$ requiring ATPase activity, which controls Na^{+} and K^{+} and loss of which in turn depress iodine transport.

The decrease in the protein binding of ^{131}I in all the experiments with alloxan diabetes, partial pancreatectomy and acetoacetic administration cannot be considered as being due solely to the effect of the decreased uptake by the thyroid gland. It has been shown in the experiments that the ratio of the plasma protein binding of ^{131}I is lower in the experimental and control than the ratio of the uptake of radio-iodine in these two groups of animals, so there is a definitive decrease in the protein binding of iodine. It is further supported by <u>in vitro</u> studies with acetoacetates. The percentage of protein binding of radio-iodine was significantly lower in the thyroid incubated with acetoacetate in the medium.

Acetoacetate and betahydroxybutyrate in the ketoacidosis, being products of an organic nature, interfere with the iodination of the tyrosine and thus bring about a reduction in the thyroxine secretion as shown by the decreased level of plasma protein bound iodine. It has been shown that acetoacetate acts like hypoglycemic sulfonylureas which induce hypoglycemia and enhance the internal insulin secretion and these hypoglycemic agents similarly show organic goitrogenic properties (147,148,149,150,151,152,153,154,155,156).

The reduction in the thyroid secretion affects the 'set point' of the hypothalamus to the blood level of the thyroid hormone and increases TSH secretion in the feed back mechanism. The increased

stimulation of the thyroid gland by TSH leads to hypertrophy and increase in the weight of the thyroid gland. The significant difference observed in the alloxan diabetic rats gives definitive evidence for the goitrogenic action of the products of hyperketonemia.

The substances characteristic of hyperketonemia are not only the factors involved in the induction of hypothyroid state but also the diminished insulin in the alloxan diabetes and after pancreatectomy could be another factor. This suggestion is supported with the experiment of partial pancreatectomy, which still showed decreased thyroid hormone secretion even in the absence of hyperketonemia and the only factor was the partial absence of pancreatic tissue or the possible diminished insulin level in the blood.

In conclusion this work has shown that the substances characteristic of hyperketonemia and diminished insulin in the pancreatic tissue are responsible for the hypo-activity of the thyroid gland; the factors affecting at various levels of uptake, protein binding of iodine and secretion of thyroxine.

Fig.8. The release of ^{131}I from the thyroid gland of alloxan diabetic rats.

↓ Intraperitoneal injection of alloxan

Fig.9a & b Blood sugar and blood acetoacetate levels 72 hours after the alloxan injection.

Fig.10 Uptake of ^{131}I calculated as counts/mg.wt. of thyroid gland in control, mild diabetic and severely diabetic rats.

A Control
B Mild diabetic
C Severely diabetic

Fig.11 The average weights of thyroid/100 g body wt. in control, mild diabetic and severely diabetic rats.

Fig.12. Plasma protein bound ^{131}I in control, mild diabetic and severely diabetic rats.

Fig.13. Blood sugar and blood acetoacetate level, 5 weeks after partial pancreatectomized and in control rats.

Fig.14. Uptake of ^{131}I expressed as counts/mg. wt. of thyroid gland in control and partial pancreatectomized rats.

A Control
B Partial pancreatectomized.

Fig.15. The average weights of thyroid/100 g body wt. in control and partial pancreatectomized rats.

Fig.16. Plasma protein bound ^{131}I in control and partially pancreatectomized rats.

Fig.17. Uptake of ^{131}I expressed as counts/mg. wt. of thyroid gland in control and diketene treated rats.

A Control
B Diketene treated

↑ 5 μci $Na^{131}I$ injection

Fig.8.

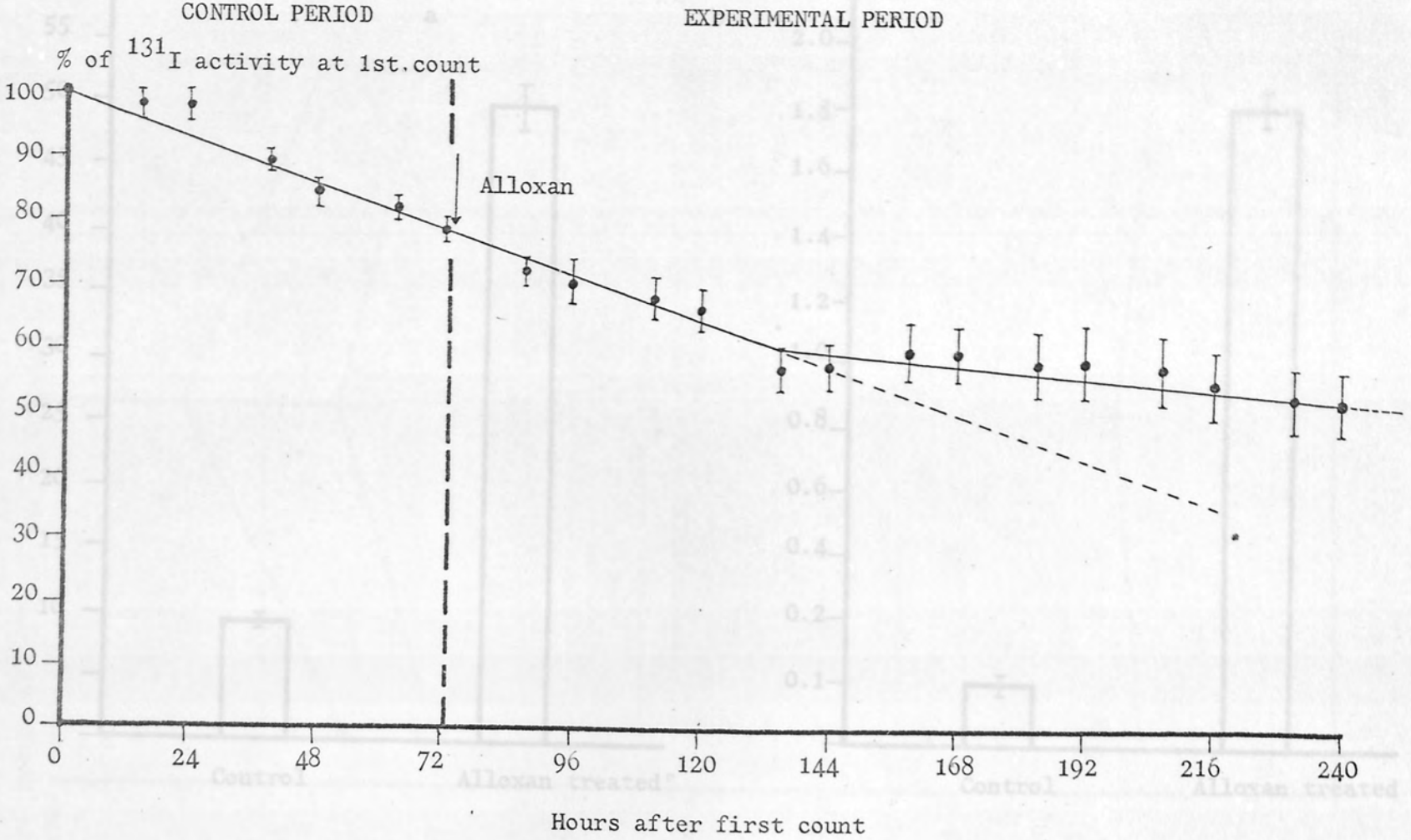
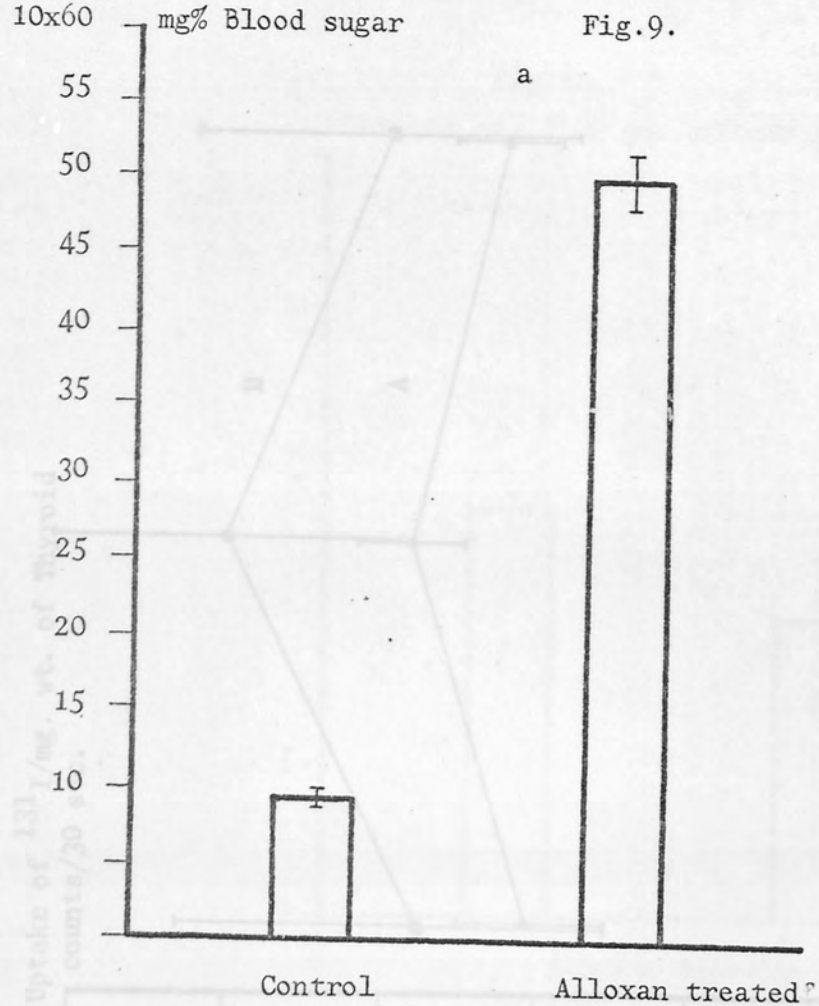

CONTROL PERIOD
EXPERIMENTAL PERIOD
% of 131I activity at 1st.count
Alloxan
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
192
216
240
Hours after first count

Fig.9.


10x60
mg% Blood sugar
a
55
50
45
40
35
30
25
20
15
10
Control
Alloxan treated

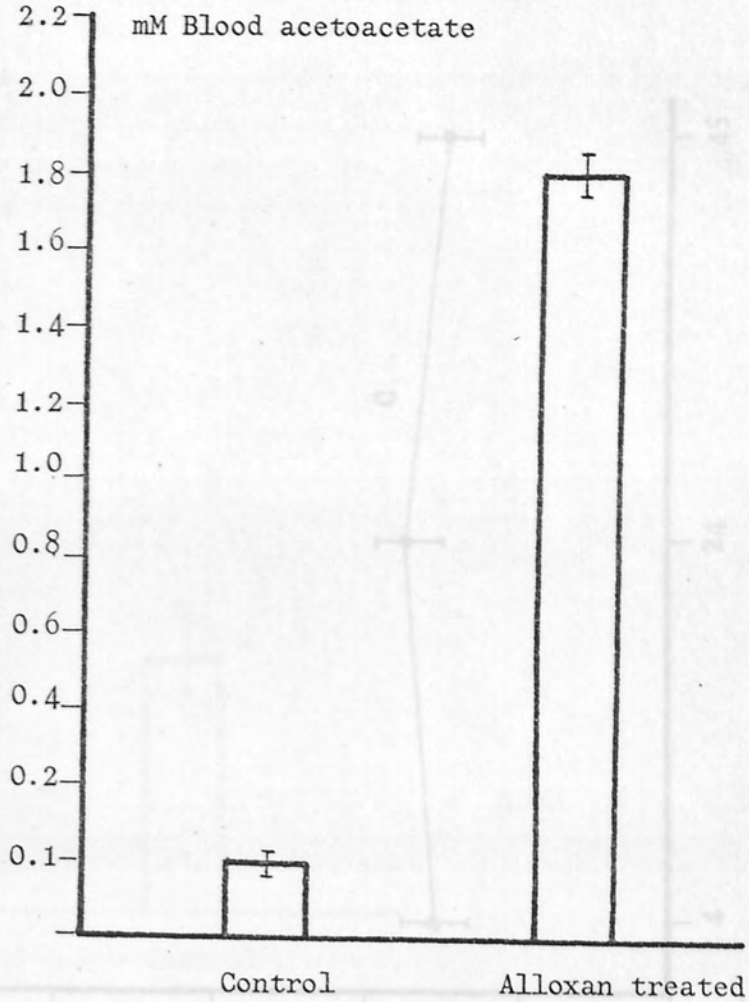

b
mM Blood acetoacetate
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.1
Control
Alloxan treated

Fig.10.

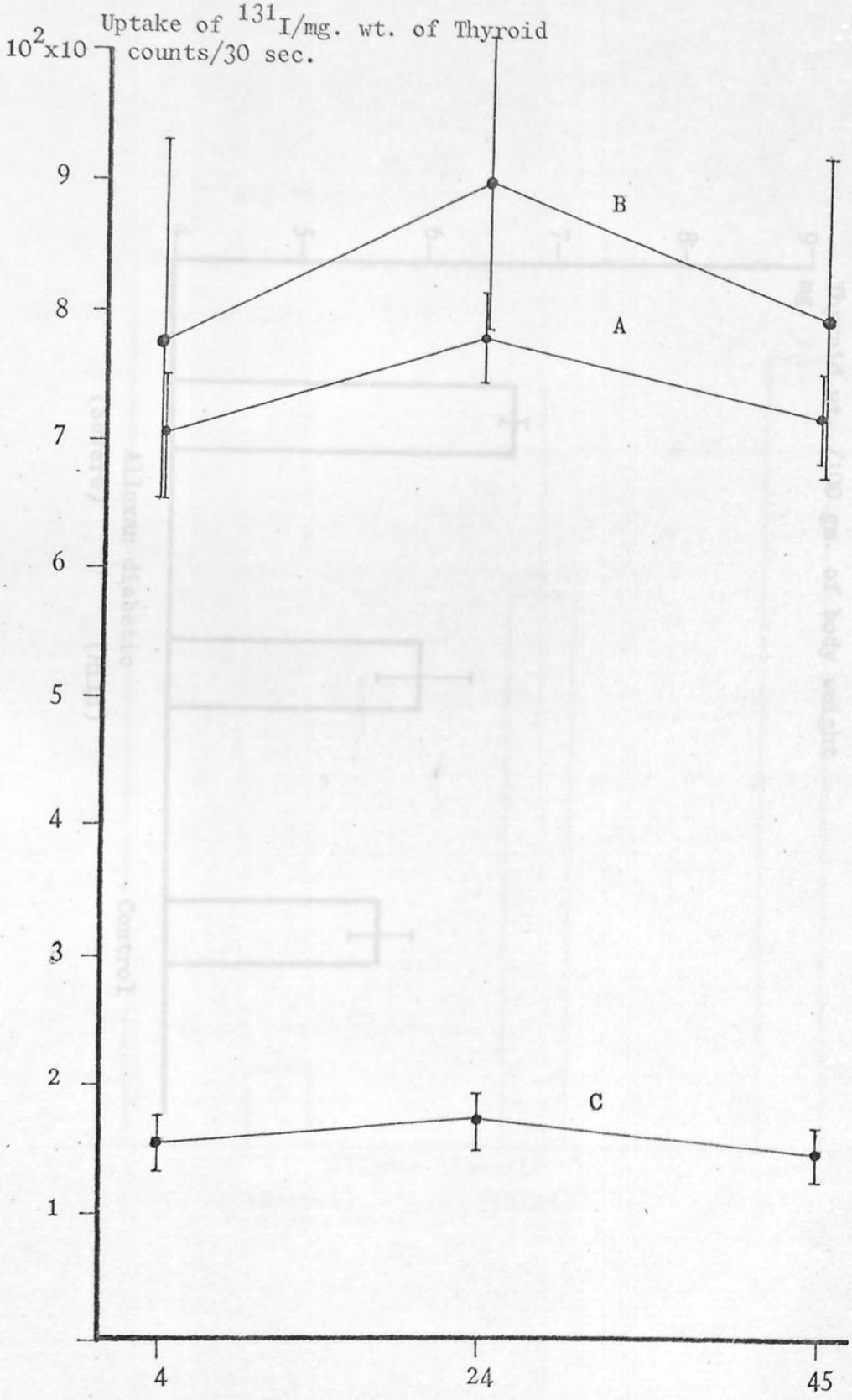

Uptake of ^{131}I/mg. wt. of Thyroid
10^2x10 counts/30 sec.
9
8
7
6
5
4
3
2
1
B
A
C
4
24
45
Hours after ^{131}I injection

Fig.11

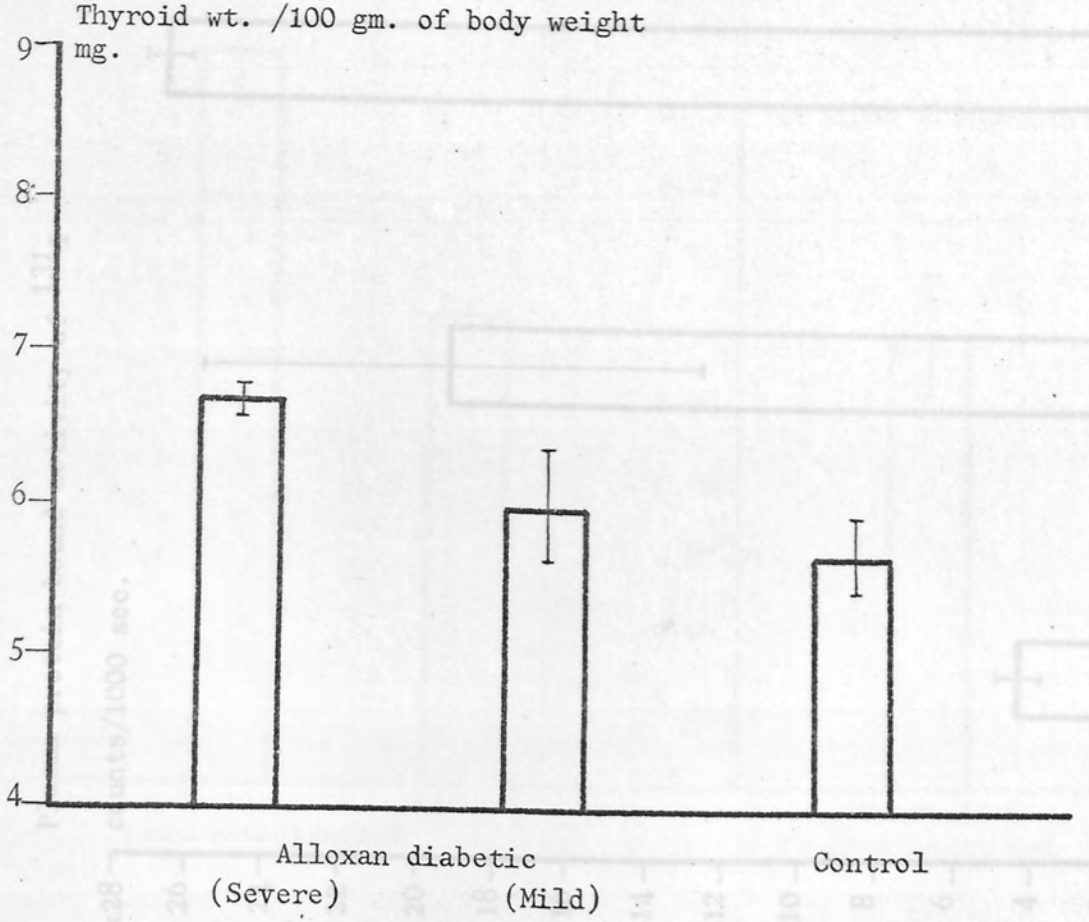

Thyroid wt. /100 gm. of body weight
mg.
9
8
7
6
5
4
Alloxan diabetic
(Severe)
(Mild)
Control

Fig.12.

Plasma protein bound activity of ^{131}I

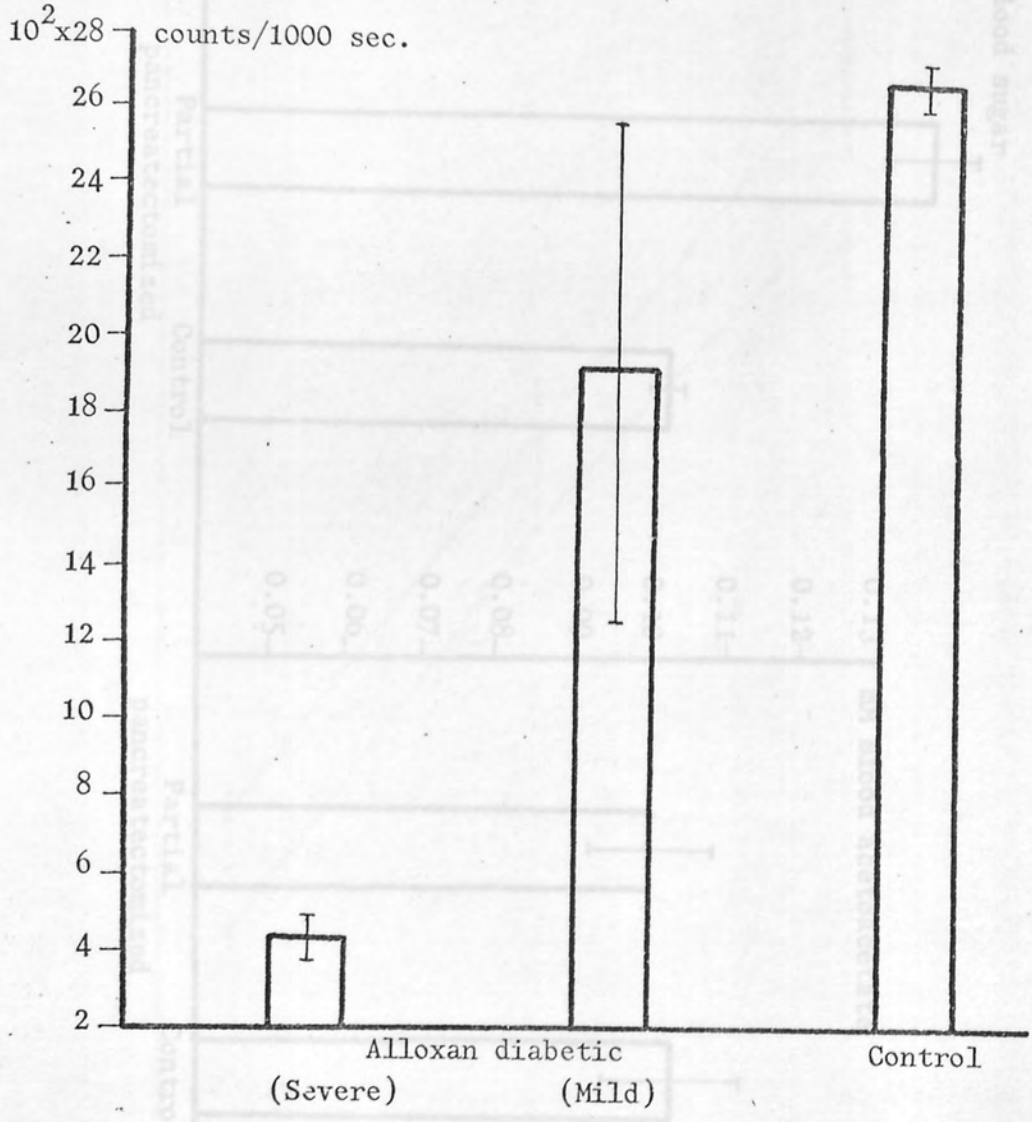


Fig.13

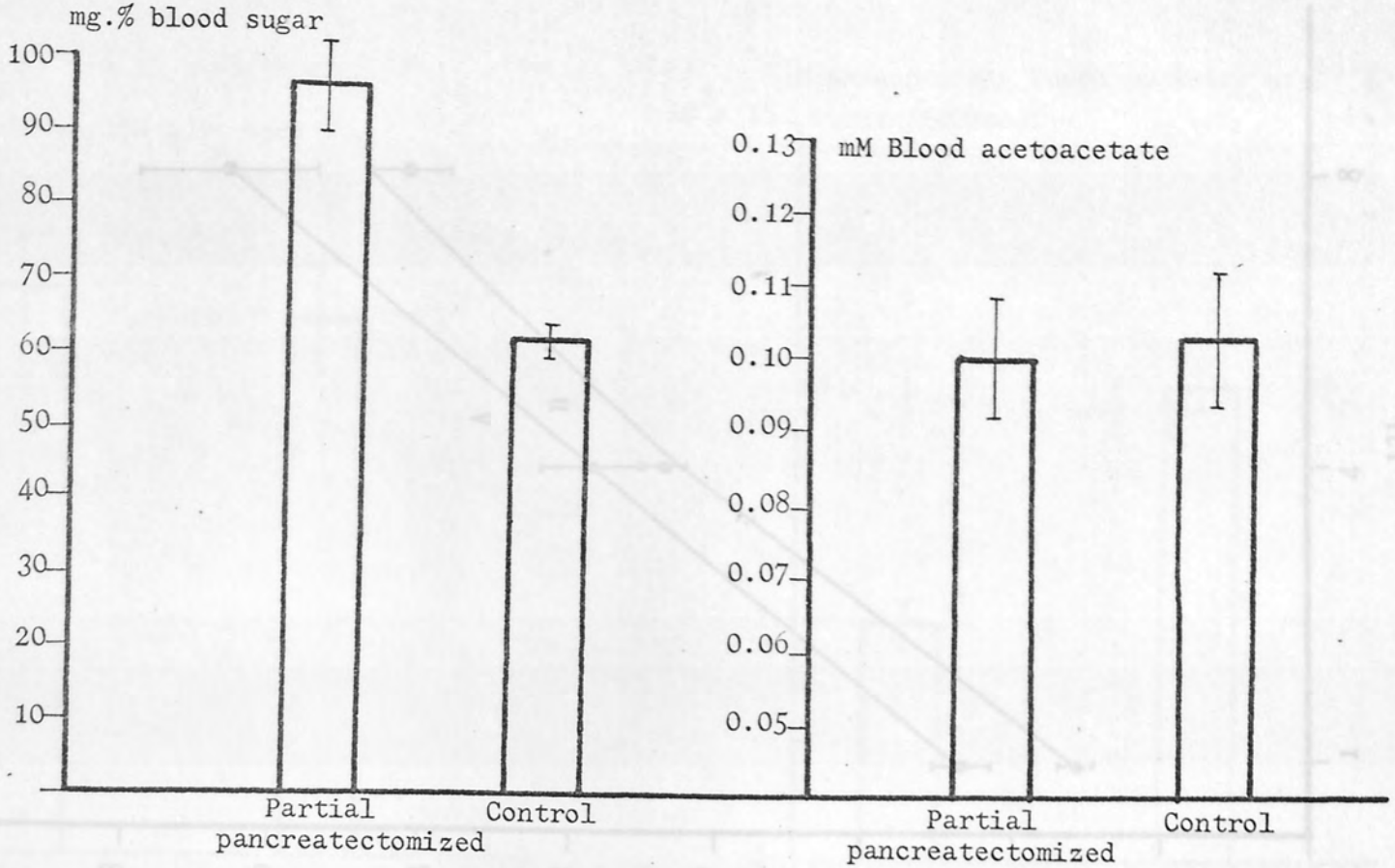

mg.% blood sugar
100
90
80
70
60
50
40
30
20
10
Partial pancreatectomized
Control
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
mM Blood acetoacetate
Partial pancreatectomized
Control

Fig.14.

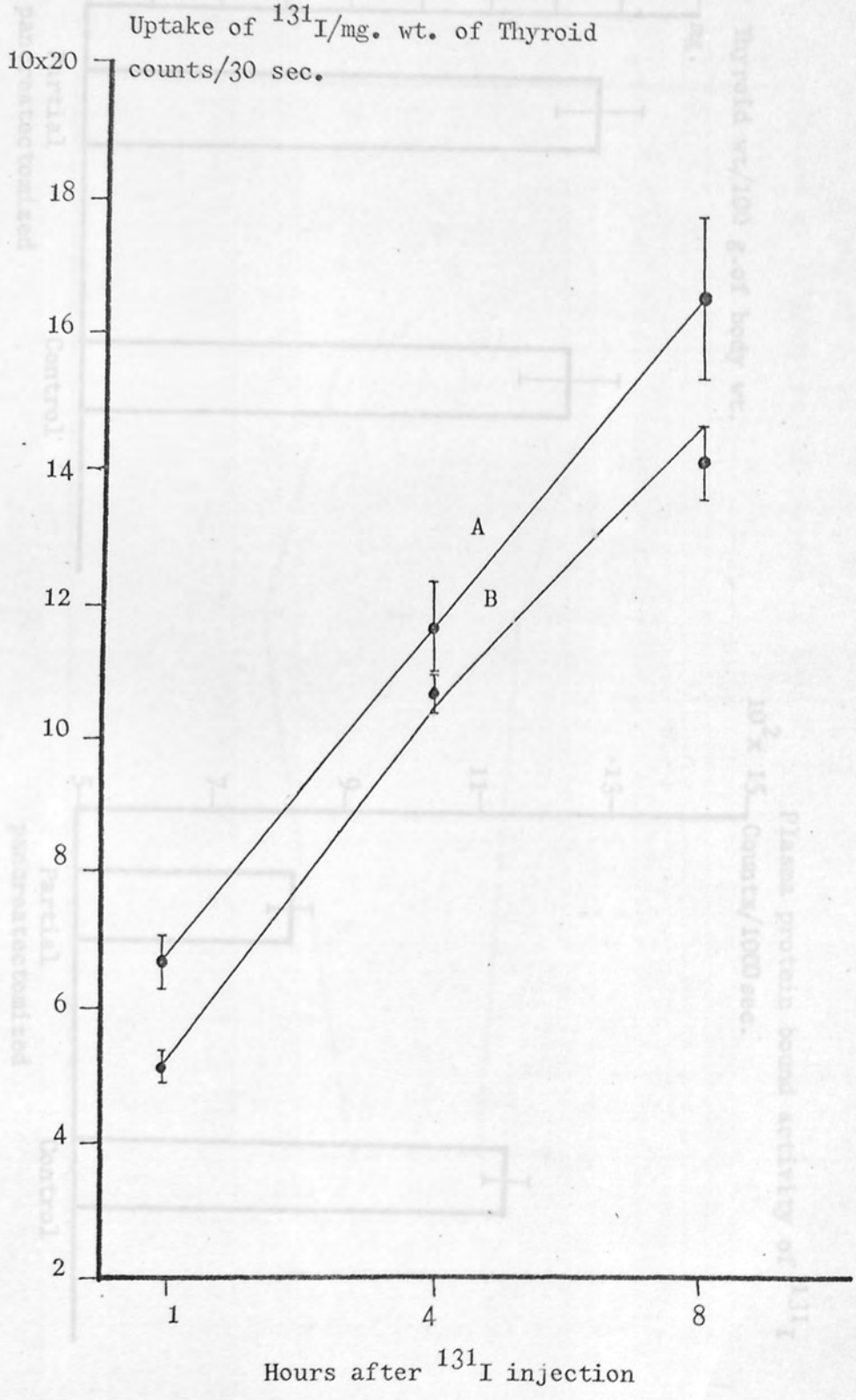

Uptake of ^{131}I/mg. wt. of Thyroid
counts/30 sec.
10x20
18
16
14
12
10
8
6
4
2
A
B
1
4
8
Hours after ^{131}I injection

Fig.15.

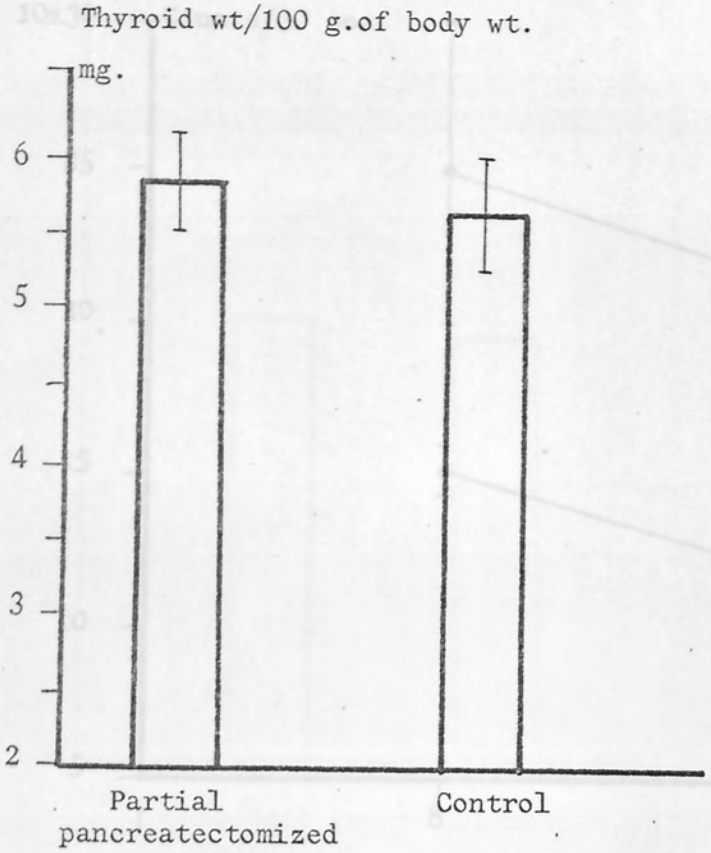

Thyroid wt/100 g.of body wt.
mg.
6
5
4
3
2
Partial pancreatectomized
Control

Fig.16.

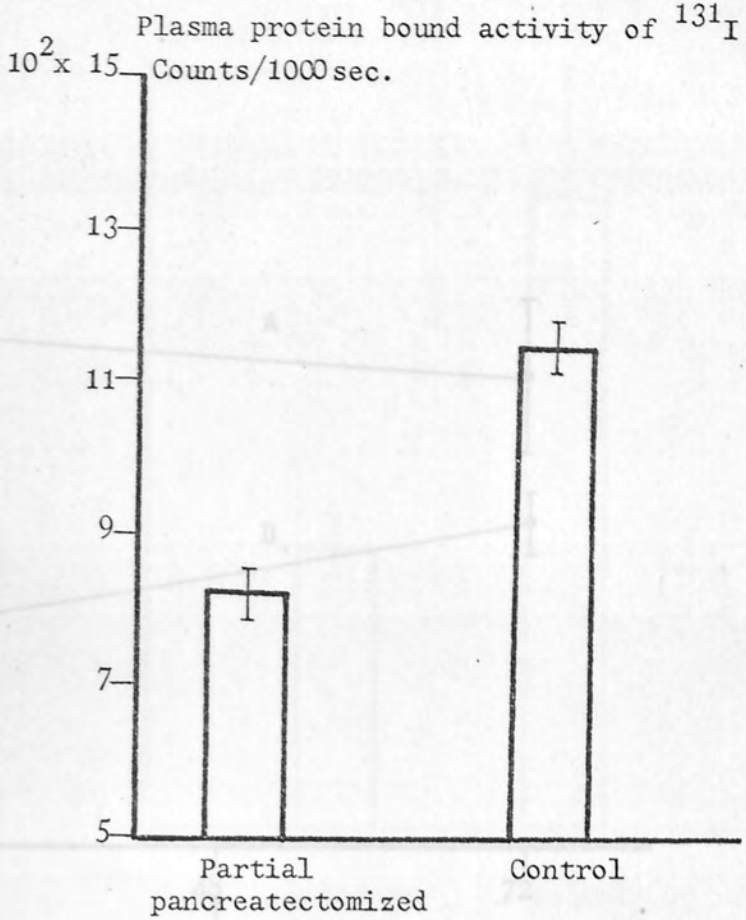

Plasma protein bound activity of ^{131}I
10^2x 15
Counts/1000 sec.
13
11
9
7
5
Partial pancreatectomized
Control

Fig.17

Uptake of ^{131}I/mg. wt. of thyroid.

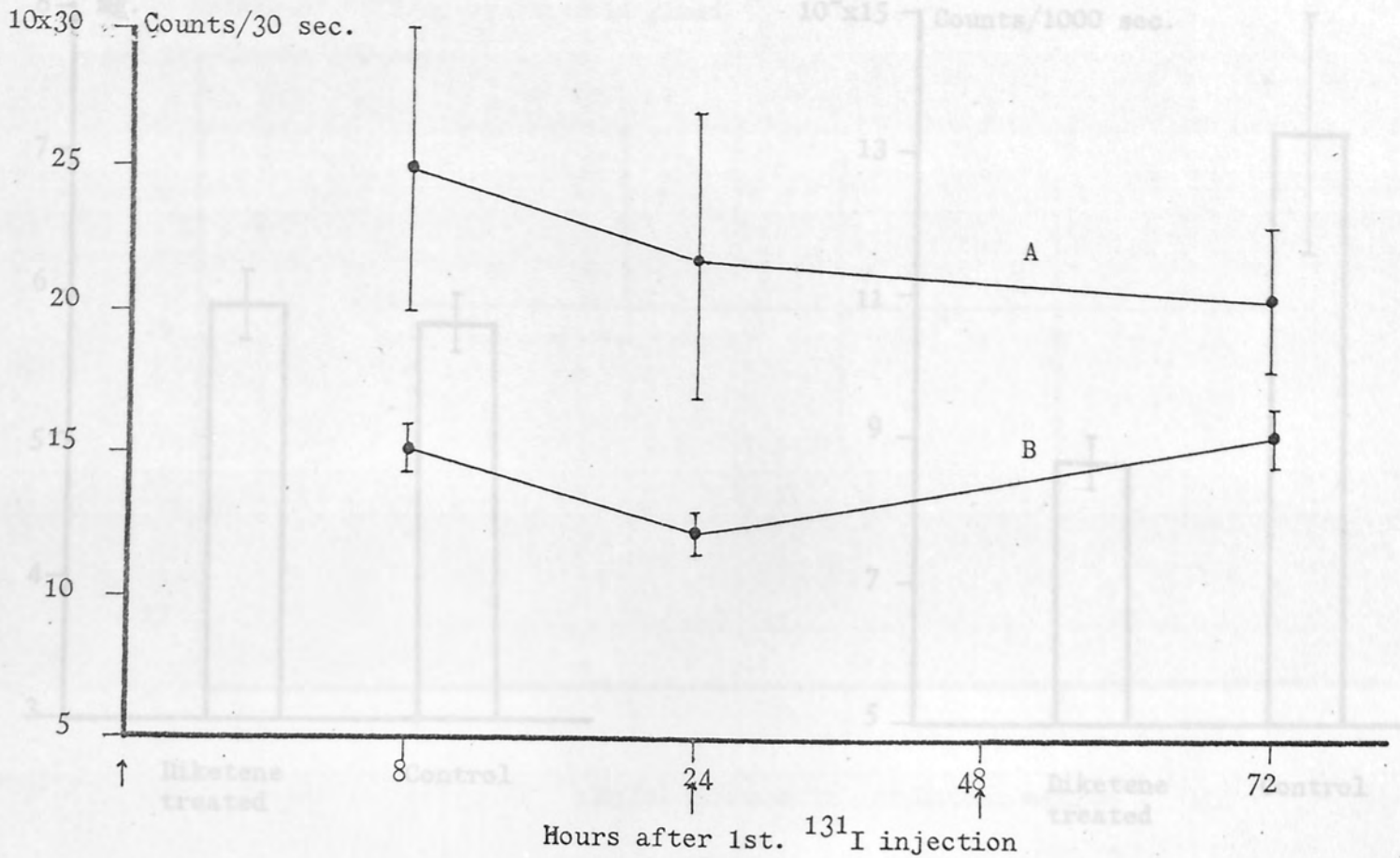


Fig.18

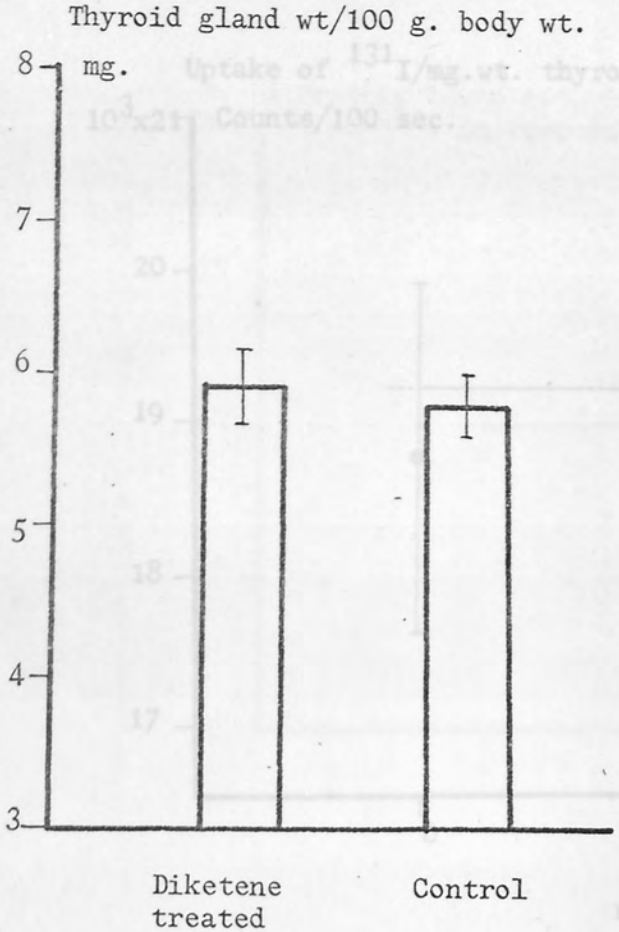

Thyroid gland wt/100 g. body wt.
mg.
8
7
6
5
4
3
Diketene treated
Control

Fig.19

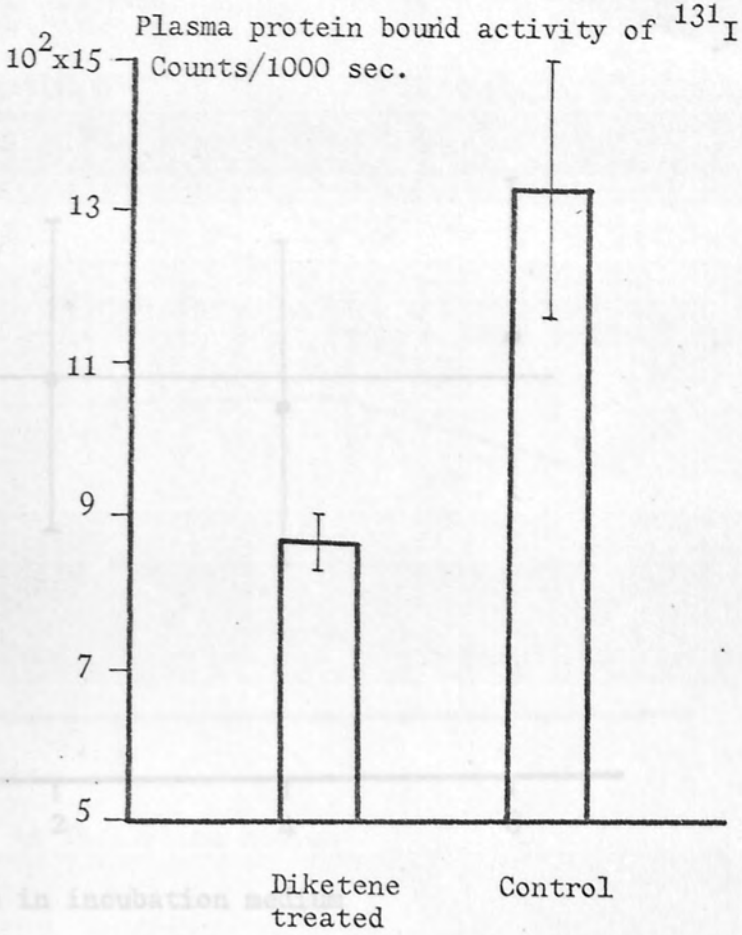

Plasma protein bound activity of ^{131}I
10^2x15
Counts/1000 sec.
13
11
9
7
5
Diketene treated
Control

Fig.20

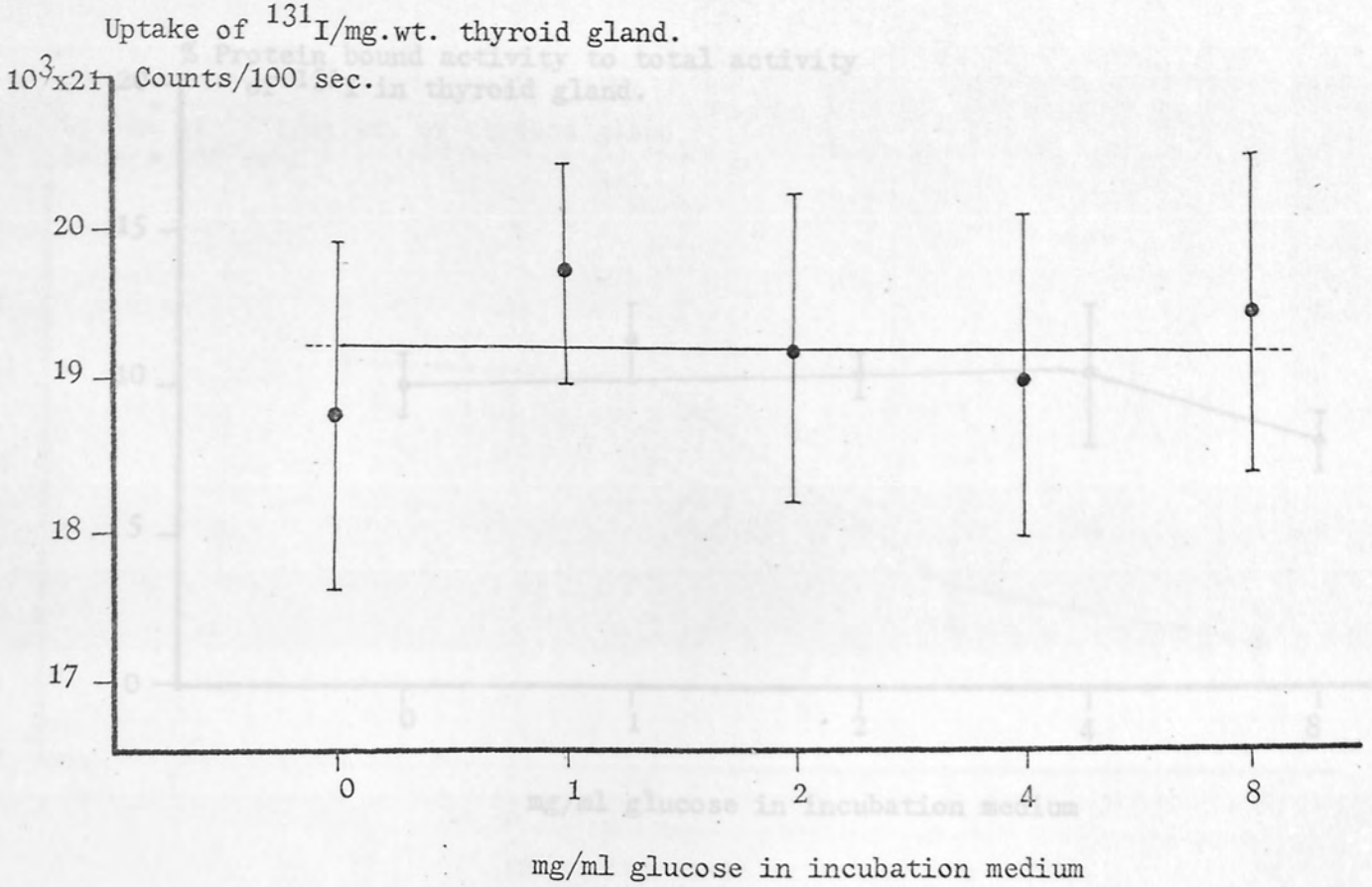

Uptake of ^{131}I/mg.wt. thyroid gland.
10^3x21 Counts/100 sec.
20
19
18
17
0
1
2
4
8
mg/ml glucose in incubation medium

Fig.21.

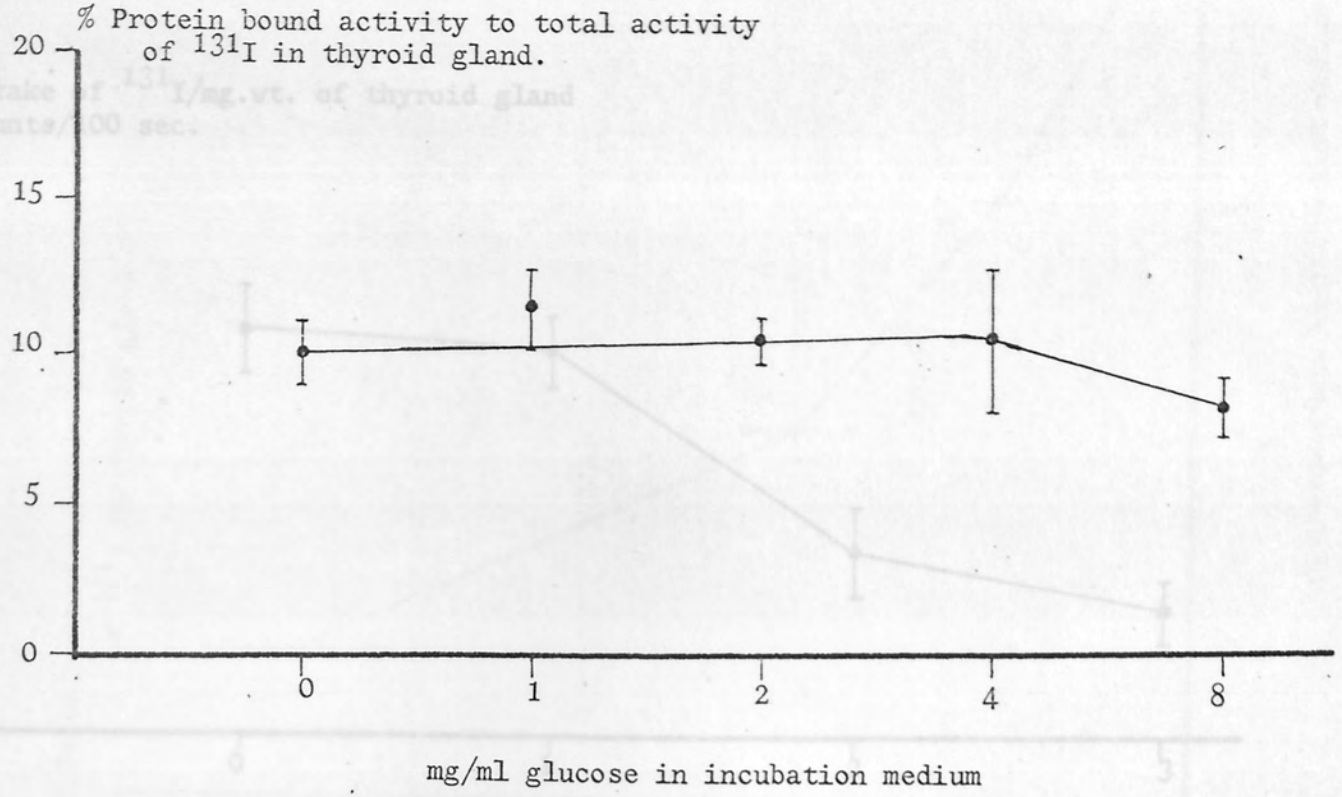

% Protein bound activity to total activity of ^{131}I in thyroid gland.
20
15
10
5
0
0
1
2
4
8
mg/ml glucose in incubation medium

Fig.22.

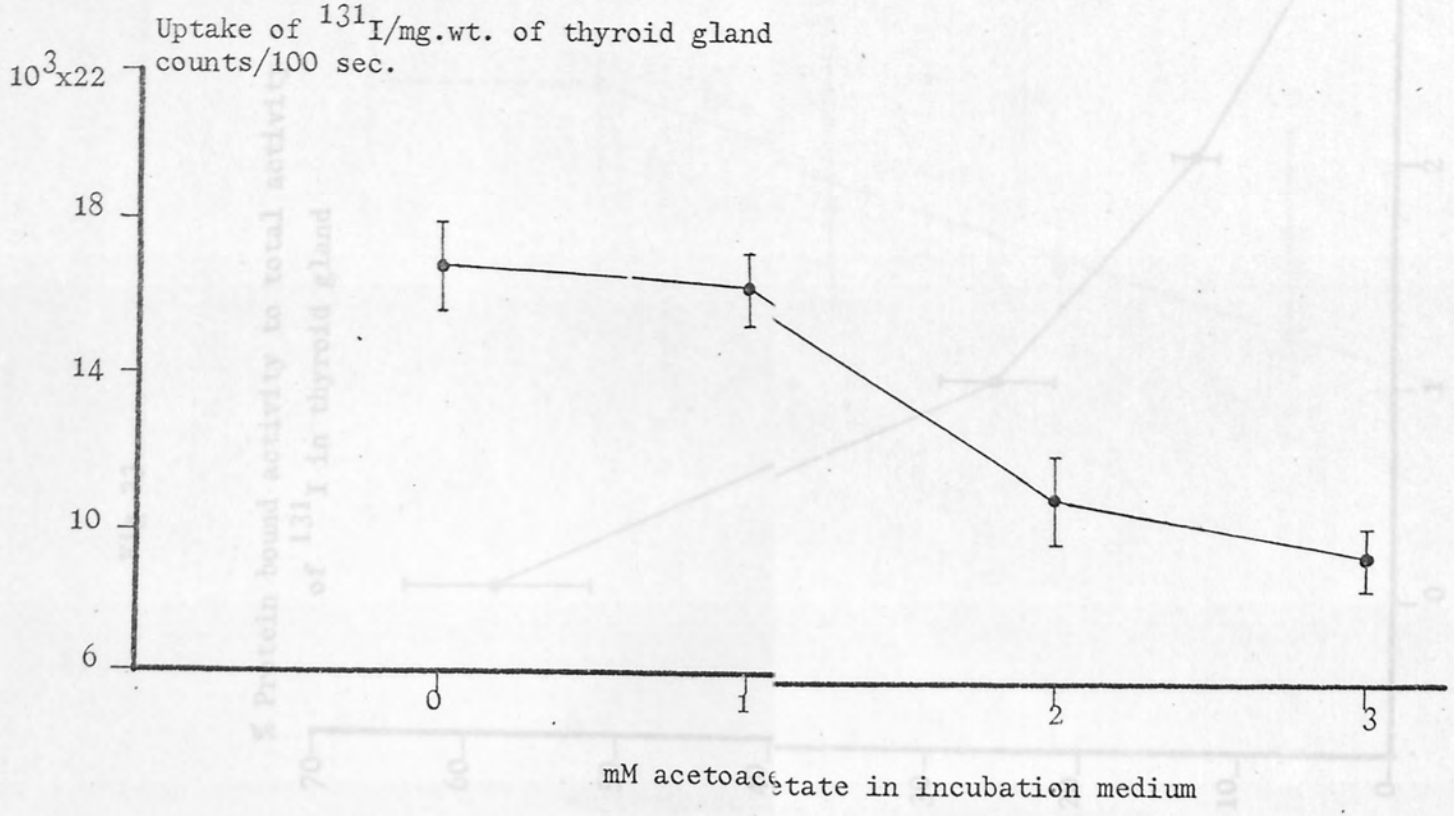

Uptake of ^{131}I/mg.wt. of thyroid gland
counts/100 sec.
10^3x22
18
14
10
6
0
1
2
3
mM acetoacetate in incubation medium

Fig.23.

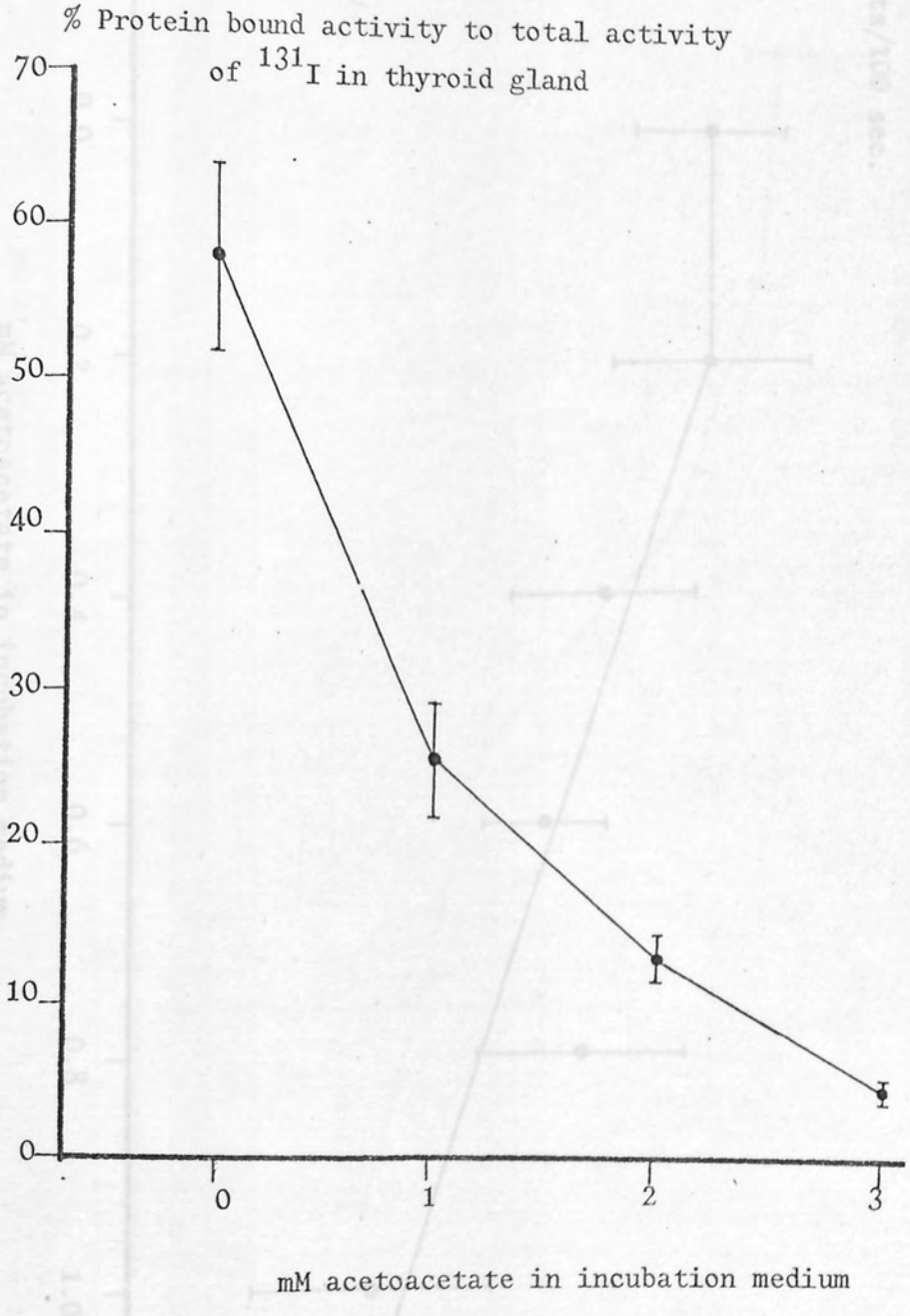

% Protein bound activity to total activity of ^{131}I in thyroid gland
70
60
50
40
30
20
10
0
0
1
2
3
mM acetoacetate in incubation medium

Fig.24.

Uptake of ^{131}I/mg.wt. thyroid gland.

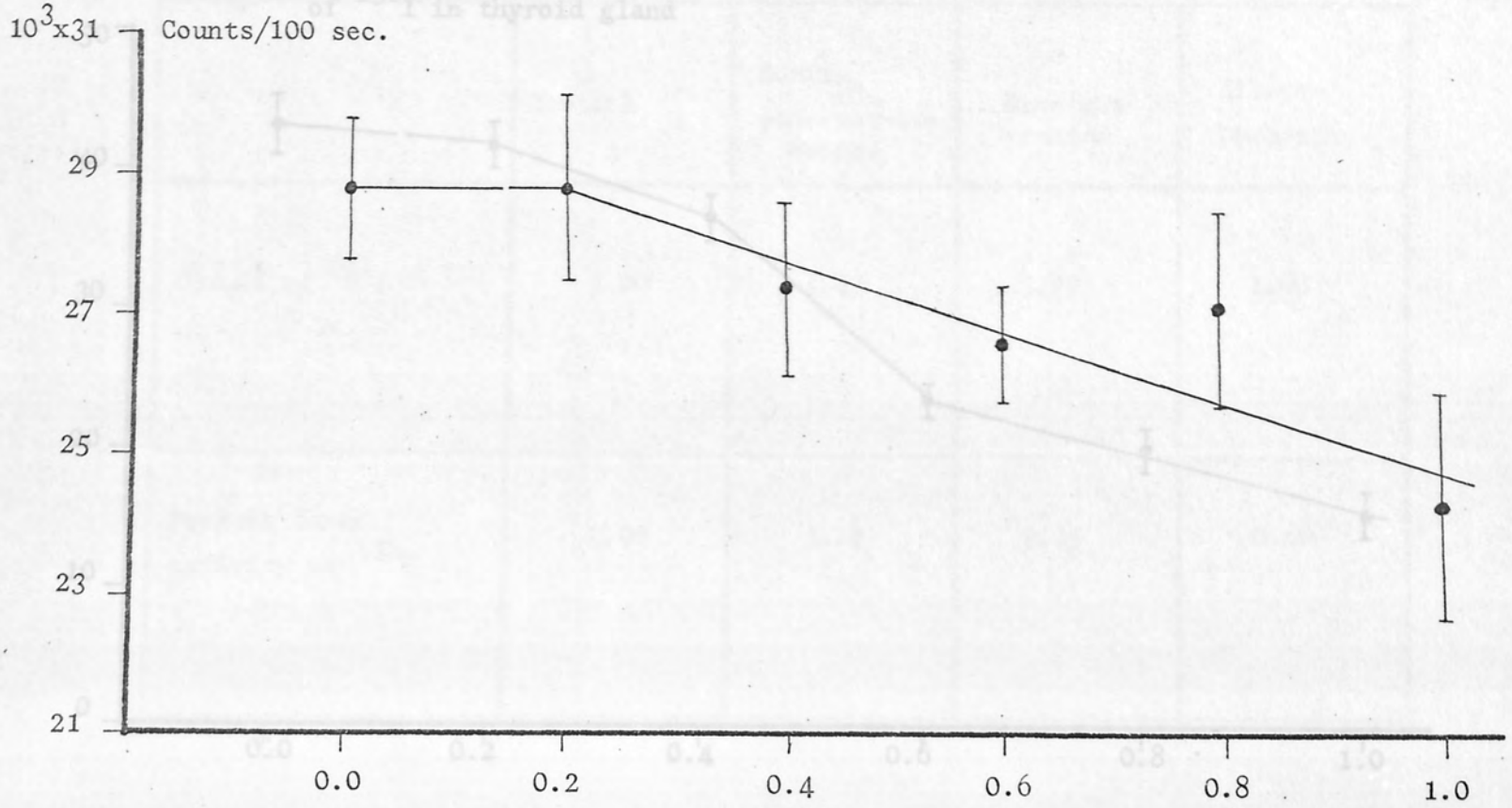


Fig.25

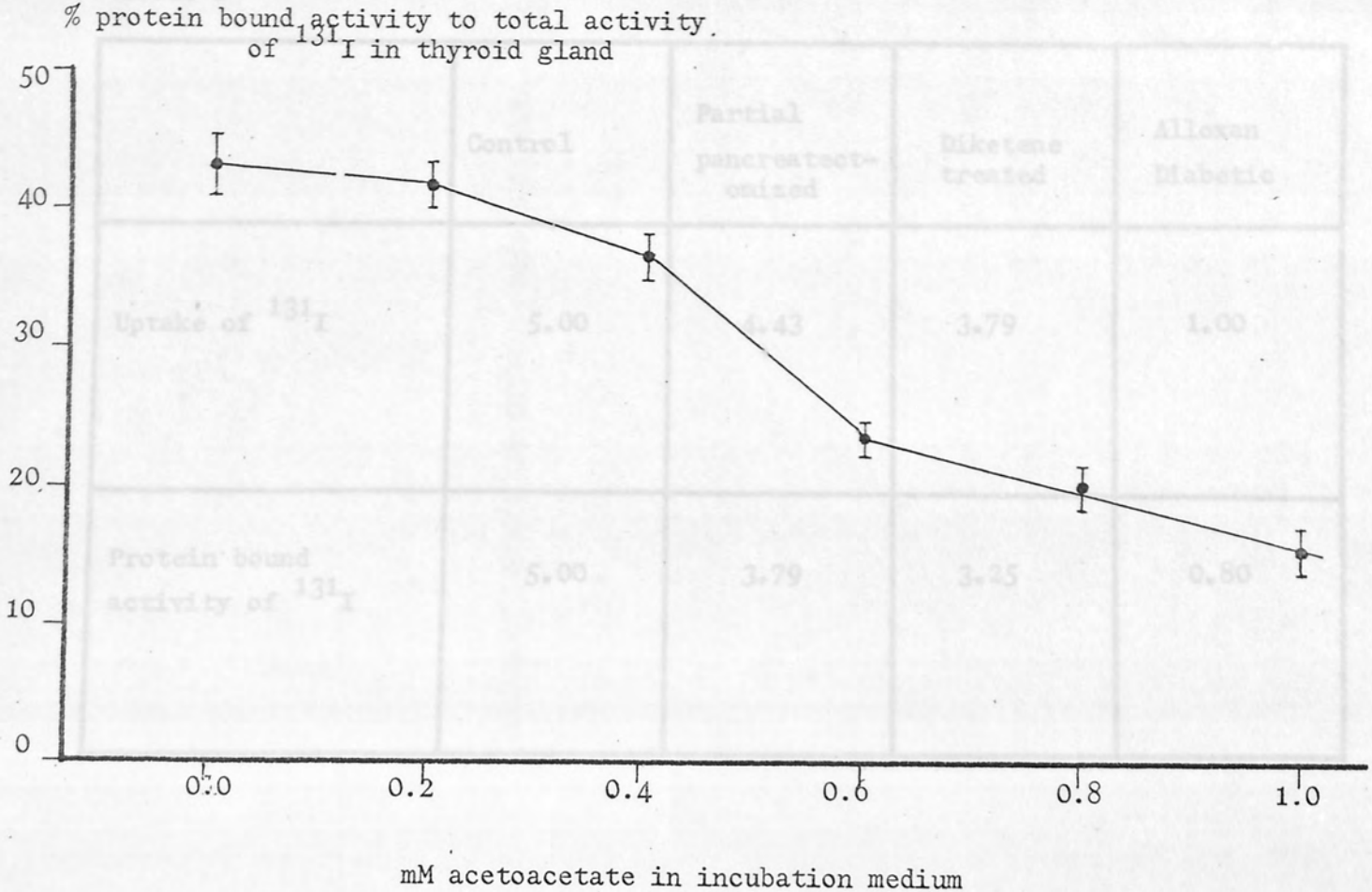

% protein bound activity to total activity of ^{131}I in thyroid gland
50
40
30
20
10
0
0.0
0.2
0.4
0.6
0.8
1.0
mM acetoacetate in incubation medium

Table 1

	Control	Partial pancreatect-omized	Diketene treated	Alloxan Diabetic
Uptake of ^{131}I	5.00	4.43	3.79	1.00
Protein bound activity of ^{131}I	5.00	3.79	3.25	0.80

EXOGENOUS INSULIN AND THYROID GLAND ACTIVITY

INTRODUCTION

In the study of insulin, metabolic changes are not only induced by the deprivation of insulin as in experimental alloxan diabetes in animals but also under conditions of excess of insulin.

Glucose uptake of isolated adipose tissue has been found to be increased by insulin (157,158). The net incorporation of fatty acids into neutral lipids by adipose tissue in vitro is enhanced by the presence of glucose in the medium and still more by the addition of insulin (159). The incorporation of the labelled amino acids is enhanced by insulin in the presence of carbohydrate substrate (160).

Glucose uptake of slices of rat brain medulla, if the donor rats are anaesthetized before sacrifice with 50% CO_2 and 50% O_2 is found to be increased by the addition of insulin in vitro (161).

Insulin added in vitro to cartilage slices from normal or hypophysectomized rats increased the incorporation of SO_4 into chondroition sulphate (162) and altered the growth of chick embryo bone (163).

Insulin stimulates the transport of glucose from plasma into the aqueous humour of the rabbit eye (164).

Insulin stimulates glucose-1-C^{14} incorporation in the liver slices from normal fed rabbits (165). The addition of insulin in vitro has been found to stimulate fatty acid synthesis (166) and amino acid incorporation (167) into liver proteins in the liver slices. Injection of insulin led to an increased rate of P^{32} turnover in various phosphate compounds of rat liver (168).

The CO_2 output is stimulated by addition of insulin to

mammary gland slices from lactating rats (169) and the fatty acid synthesis is stimulated after the onset of lactation (170,171).

In chick heart explants insulin caused an increase in incorporation of P^{32} into RNA - P (172).

Insulin enhanced the conversion of glucose C^{14} to $C^{14}O_2$ and lipid in slices of rat anterior pituitary gland (173).

In the culture of mouse skin, the number of mitosis increases with insulin (174).

Addition of insulin to culture medium enhances the incorporation of orotic acid C^{14} into RNA in the mouse uterus organ culture (175).

The experiments involving the deprivation of thyroid gland activity cannot be performed satisfactorily in an isolated unit in the intact animals. The deprivation of insulin in the intact animal with induction of experimental diabetes leads to various other changes in the metabolism like the production of hyperketonemia which themselves interfere with thyroid gland activity already discussed in the previous chapter.

Partial pancreatectomy is the closest possibility of inducing a condition of partial insulin deprivation and an absence of the substances characteristic of hyperketonemia which interfere with the thyroid gland activity. Even this partial deprivation is not independent of other factors. Its induction in the partial pancreatectomy not only involves the removal of beta cells, the source of insulin, but also the alpha cells and the exocrine pancreatic tissue.

The induction of excess of insulin by exogenous administration is another approach to the study of its effect. Effects seen from excess insulin might be interpreted as a mode of manifestation of a control mechanicm of endocrine pancreas on thyroid gland activity.

The work described here is an attempt to study the different aspects of thyroid gland activity under the induced conditions of excess of insulin by exogenous administration.

MATERIALS AND METHODS

Preparation of Insulin

A stock solution of insulin was prepared by dissolving 1 mg. of crystalline hormone in a minimum amount of 0.003 N/HCl and diluting the solution to 1 ml. It was sterilised by passing through a millipore filter with an average porosity of 0.45 μ.

For in vivo preparation, just before the administration, the stock solution was mixed with millipored phosphate buffer of pH 8.4 to provide the required concentration.

For in vitro preparation, just before the cultures were set up the stock solution was mixed with basal medium to provide the required concentration.

Few doses of insulin

Eight rats weighing between 200 and 220 gms. were used. Each rat was injected with 10 μci of radio-iodine intraperitoneally. The rate of the release of radio-iodine from the thyroid gland was determined. The neck region of the thyroid gland was counted for six times in the control period. Immediately after the seventh, eighth and ninth counting 1 I.U./Kg. body wt. of insulin was injected subcutaneously. The rate of the release of radio-iodine was determined on 18 occasions by counting the neck region of the thyroid gland.

Blood samples of the rats for the estimation of blood sugar levels were taken, 24 hours before and 3 hours after the first injection and 48 hours after the last injection of insulin.

Many doses of insulin

Eight of the fourteen rats weighing between 200 and 230 gms. were used. Each rat was injected with I.U./kg. body wt. of insulin for 20 days. The daily injection was given at mid-day. Some rats showed slight hypoglycemic symptoms; these were not treated with glucose as they recovered eventually. The other six rats were administered with only phosphate buffer of pH 8.4 at the same time and for as many times as the animals with insulin and considered as controls. The injections were carried out for 20 days.

24 hours after the last injection of insulin, each rat was injected with 5 µci of ^{131}I ($Na^{131}I$). The uptake of radio-iodine by the thyroid gland was determined at the 1st., 4th and 8th hour after the radio-iodine injection. After 48 hours, the rats were killed by a blow on the head and 5 ml. of venous blood was taken from each rat. The thyroid gland was taken out, cleaned of the fatty tissue and weighed carefully in a sensitive balance. The blood samples were used for the estimation of plasma protein binding of ^{131}I.

In vitro insulin

(a) Secretory rate

30 of T-O strain mice were kept on low iodine diet for a week and then injected with 0.2 µci of ^{131}I($Na^{131}I$). After six hours the animals were killed by a blow on the head and the thyroid glands excised out and incubated for the study of the release of radio-iodine. The dishes containing the 15 experimental thyroids had added to them 100 µU./ml. of insulin in the incubation medium. The glands were incubated for 18 hours. The activity of ^{131}I left in the thyroid gland and released into the incubation medium was

measured.

The above experiment was further performed with slight modification to increase the sensitivity of the ratio of the release of radio-iodine from the experimental and control thyroids. The thyroid glands on the 30 mice labelled in vivo were incubated for 12 hours in the normal medium. This medium was discarded and the thyroids were transferred to a fresh lot of dishes, in which 15 had normal incubation medium as control and in the rest the incubation medium contained 100 μU/ml. of insulin. The thyroids were incubated for a further 12 hours. The activity of radio-iodine left in the thyroid gland and released in the medium was estimated.

(b) Uptake and protein binding.

The concentrations of 25,50,100 and 200 μU/ml. of insulin in the incubation medium were used for 8, 8, 9 and 5 isolated thyroid glands of the mice, while 20 thyroids were incubated in the normal incubation medium and considered as controls.

At first all the thyroids were incubated in the normal medium for 6 hours. After,these were transferred to the fresh lot of dishes with the above mentioned varying concentrations of insulin and incubated for a further 12 hours. 0.2 μci of ^{131}I ($Na^{131}I$) was added to each dish. The incubation was continued for a further 6 hours.

Uptake of radio-iodine was determined and thyroids were carefully weighed in a sensitive balance. The thyroids were treated for the determination of the activity of the protein bound ^{131}I.

RESULTS

Few doses of insulin

Fig.26 shows the release of ^{131}I from the thyroid gland, throughout the period of the experiment. An almost regular release of radio-iodine was observed in the control period. The effect of insulin was observed. Immediately after the treatment, there was stimulation of the release of radio-iodine. The stimulation of the release of radio-iodine remained persistent for 24 hours after the last injection of insulin. The sudden inhibition of the release of radio-iodine was observed for nearly two days and finally a return to the normal rate as previously observed in the original control period.

As shown in Fig.27 the blood sugar level was quite normal 24 hours before the first injection of insulin. Considerable hypoglycemia was induced three hours after the insulin injection. 48 hours after the last injection the blood sugar level had returned to almost the original level seen before the insulin administration in the original control period.

Many doses of insulin

Fig.28 shows the uptake of ^{131}I expressed as counts/mg. wet weight of the thyroid gland. The uptake of radio-iodine was significantly higher at the 1st. hour but there was no difference at the 4th and 8th hours in insulin treated and control rats.

In Fig.29 the average of the weights of the thyroid gland per hundred gms. of the body weight is shown. The thyroid weight of insulin treated rats failed to show any difference from the average weights of the thyroid of control rats.

In Fig.30 the average of the plasma protein bound ^{131}I ($PB^{131}I$)

levels is shown. The level in the insulin treated animal has significantly increased over the level of the control animals. ($P > 0.001$).

In vitro Insulin

(a) Secretory rate

Fig.31 shows the in vitro secretory rate of radio-iodine from the incubated thyroid gland. Insulin failed to show any effect on the release rate of radio-iodine from the thyroid gland even with the modification to increase the sensitivity.

(b) Uptake and Protein binding

Fig. 32 shows the uptake of ^{131}I calculated as counts/mg.wet weight of thyroid gland. Insulin failed to show any effect on the uptake, even with the dose as high as 200 μU/ml. in the incubation medium.

Fig.33 shows the percentage protein binding of ^{131}I in the thyroid gland. Insulin had a progressive increase in the protein binding of ^{131}I in the gland and even a small dose of 25 μU/ml. in the incubation medium shows a significant increase in the protein binding of ^{131}I above that seen in the thyroids of the control medium.

DISCUSSION

Insulin failed to show any significant difference both in the in vivo and in the in vitro experiments on the uptake of ^{131}I by the thyroid gland. It is well known that insulin is concerned with the transfer of glucose across cell membranes or across interfaces which separate the extracellular phase from the sites of enzymatic action (176). This could conceivably involve physical problems of permeability but need not necessarily do so since it is recognised that enzymatic or chemical reactions might be concerned in such

transport. However, it seems that insulin does not affect the transport of iodine directly or indirectly through the membrane of the thyroid gland.

In the in vivo experiments, the results of the increase in the release rate of ^{131}I from the thyroid and increase in the plasma protein bound ^{131}I show that insulin stimulates the release of iodine hormone from the thyroid gland. In the in vitro experiments insulin failed to stimulate the release of radio-iodine from the thyroid gland labelled in vivo. This shows that mobilisation of the iodine hormone from the thyroid is not due to the direct action of the insulin on the gland, instead the action is mediated through an indirect axis.

The activity of the thyroid gland is under the direct control of thyrotrophin from the anterior pituitary gland. In the maintenance of homeostatic balance, the thyroxine secreted acts in a servo-mechanism to reduce the production or release of TSH. This action of thyroxine may be on the pituitary directly or via the hypothalamus.

The hypothalamus appears to play some role in the regulation of TSH release and it is apparent that the thyroid gland activity that follows the injections of TSH can also be achieved by stimulation of the hypothalamus (177). Certain hypothalamic lesions either impair or inhibit TSH secretion. Such lesions may inhibit the goitre formation that normally results from administration of antithyroid compounds. These lesions also interfere to some degree with normal thyroid function (178).

The involvement of the pituitary gland in the action of insulin on the thyroid gland seems to be apparent from the results of the in vivo experiments with few doses of insulin. Insulin immediately

stimulated the release of hormone from the thyroid gland and enhanced the level of thyroxine in the blood. It could be suggested that increased thyroxine levels in turn might have acted in the feedback mechanism to inhibit thyrotrophin secretion. The inhibited thyrotrophin secretion resulted in the further inhibition of the thyroid hormone secretory rate. This was clearly observed in the experiment where the release of radio-iodine or thyroxine was suddenly inhibited after the increased release prior to it. However, the release rate returned to normal, which might be the normalisation of the thyroxine level in the blood and subsequently the normal secretion of thyrotrophin further rehabilitated the normal release rate of the thyroid hormone.

The increase in the thyroxine levels in the blood with stimulated release of radio-iodine is supported by the increased plasma protein bound ^{131}I shown in the in vivo experiments with many doses of insulin. The plasma protein bound iodine is direct evidence of the thyroidal hormone level as it has been shown that thyroxine added to serum becomes rapidly and finally attached to protein (179,180,181,182).

Further evidence for the involvement of the pituitary gland in the control of the regulation of the activity of the thyroid gland through TSH in the feedback mechanism could be further evaluated from the in vivo experiment of many doses of insulin. The daily administration of insulin should have resulted in the continuous hyperactivity similar to hyperthyroidism if considered irrespective of the involvement of thyroid-pituitary feed back mechanism and induced decrease in the weight of the thyroids of the animals treated. It has been shown that the weight of the thyroid gland is decreased in hyper-

thyroidism (183). The average of the weights of the insulin treated rats, however, failed to show any difference from the average of the weights of the control rats. This shows that there must have been some sort of mechanism which might have been inhibiting the continuous stimulating effect of insulin and this seems to be the pituitary gland operating through the regulation of T.S.H. Conclusively it seems quite probably that somehow or other insulin stimulates the thyroid secretory rate through the pituitary axis.

It is a big query whether the action of insulin is directly on the pituitary gland or mediated through the hypothalamus. It has been shown that the thyrotrophin release factor transported to the pituitary controls the T.S.H. release in response to altered thyroid hormone levels (77). However, there is a possibility of the pituitary gland being directly involved in the thyroid hormone release control, for it has been found that insulin enhanced the conversion of glucose C^{14} to $C^{14}O_2$ and lipid in the slices of the rat anterior pituitary in vitro (173).

In vitro experiments with insulin showed an increase in protein binding of ^{131}I in the thyroid gland. It has been shown that under aerobic conditions (50,51,52), iodide is oxidised by an enzyme 'Tyrosine iodinase' which transfers iodine to available tyrosine in the follicle (53,54). It had been suggested from certain findings that insulin directs ATP from its function in maintaining a barrier against glucose entrance into other reactions, which favours the synthesis of peptides (183,184) and raises the energy level of the cell (185).

It could be suggested that the increase shown in the protein

binding of ^{131}I in the thyroid gland may be due to the rise in the energy level of the cell through raising the activity of the enzyme 'Tyrosine iodinase' and subsequently increased iodine incorporation with the protein in the thyroid gland or ATP is directly promoting the iodine binding into the protein and coupling of iodinated proteins in the formation of thyroxine. However, an inhibitor of this enzyme action appears to be necessary which may act as a control on hormone synthesis in the thyroid of intact animals.

In conclusion it could be suggested that insulin stimulates the thyroid gland activity through the hypothalamus and TRF or directly by stimulating the pituitary. This stimulates thryoid hormone production in the thyroid gland and the secretion of produced hormone, the mechanism of the control of hormone release or production being by feed back mechanism involving the thryoid, hypothalamus and pituitary glands.

Fig.26. The release of ^{131}I from the thyroid gland of insulin treated rats.

↓ Intraperitoneal injection of insulin

Fig.27. Blood sugar levels at the different times in the above experiment.

Fig.28. The uptake of ^{131}I expressed as counts/mg.wt. of thyroid gland in control and insulin treated rats.

A Insulin treated

B Control

Fig.29. The average weights of thyroid/100 g body wt. in control and insulin treated rats.

Fig.30. Plasma protein bound ^{131}I in control and insulin treated rats.

Fig.31.a & b. <u>in vitro</u> release rate of ^{131}I from the thyroid gland of the mouse incubated in control and insulin added culture medium.

Fig.32. Dose response of insulin on the uptake of ^{131}I expressed as counts/mg. wt. of thyroid gland of the mouse incubated in the culture medium.

Fig.33. Dose response of insulin on the protein binding of ^{131}I in the thyroid of the mouse incubated in the culture medium.

Fig.26.

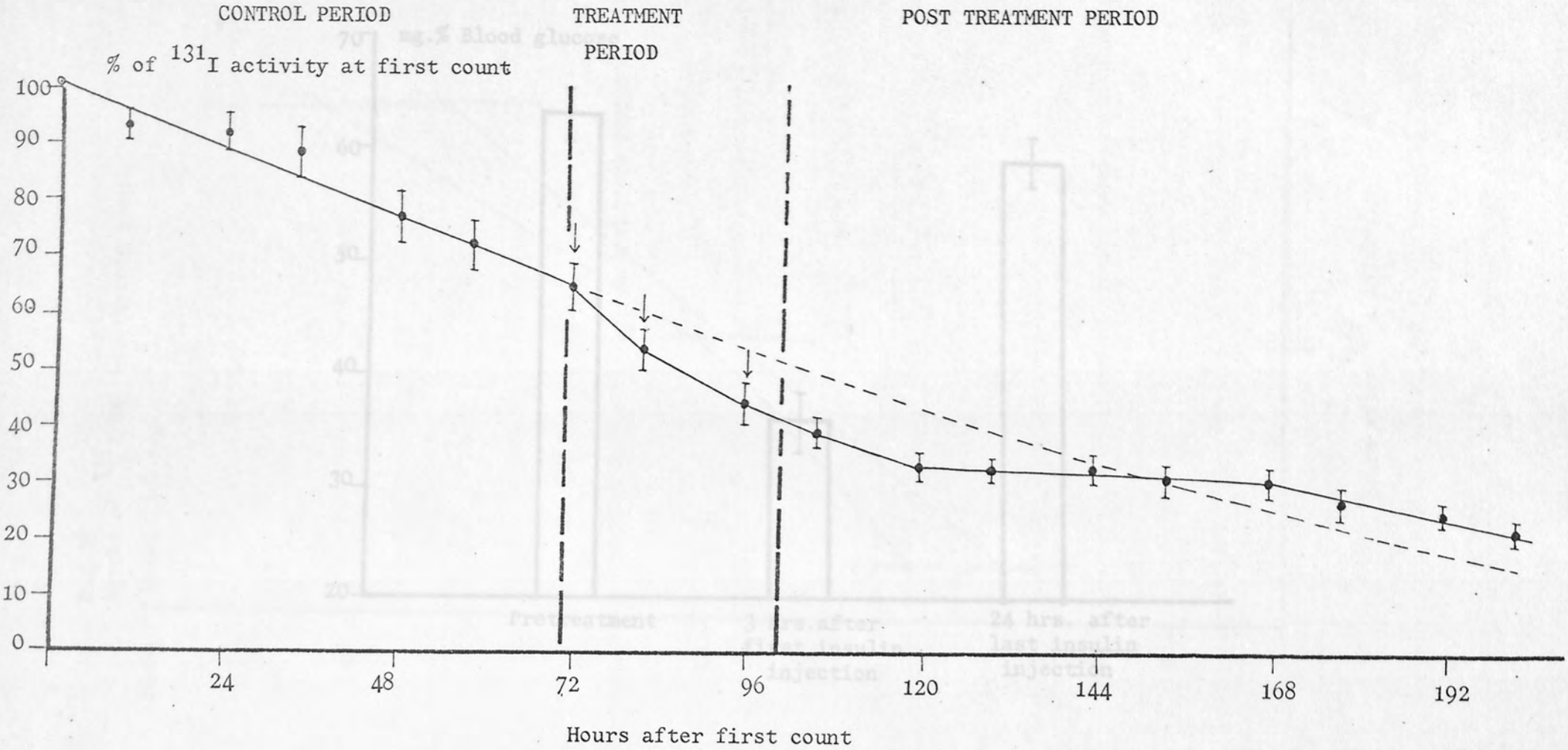

CONTROL PERIOD
TREATMENT PERIOD
POST TREATMENT PERIOD
% of 131I activity at first count
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
120
144
168
192
Hours after first count

Fig.27

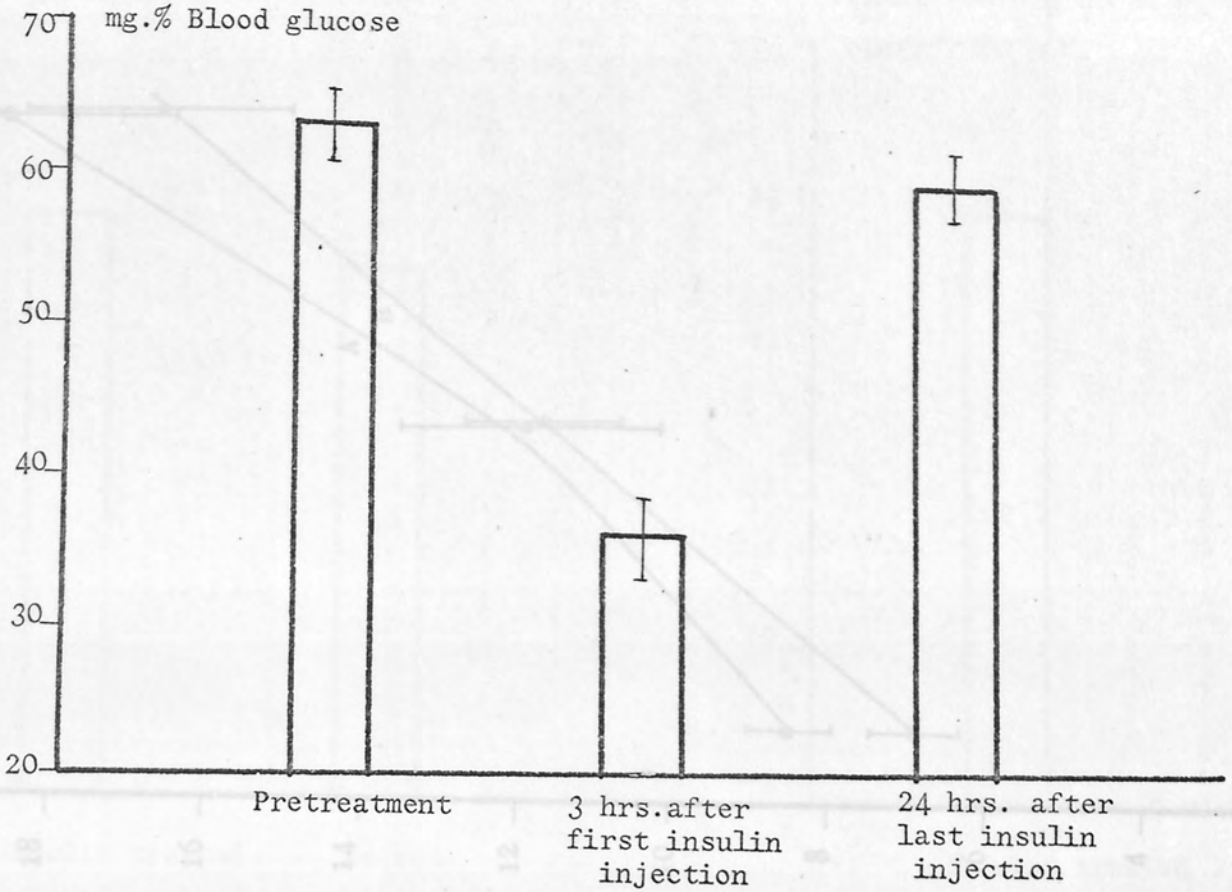

mg.% Blood glucose
70
60
50
40
30
20
Pretreatment
3 hrs.after first insulin injection
24 hrs. after last insulin injection

Fig.28
Uptake of ^{131}I/mg. wt. thyroid gland.

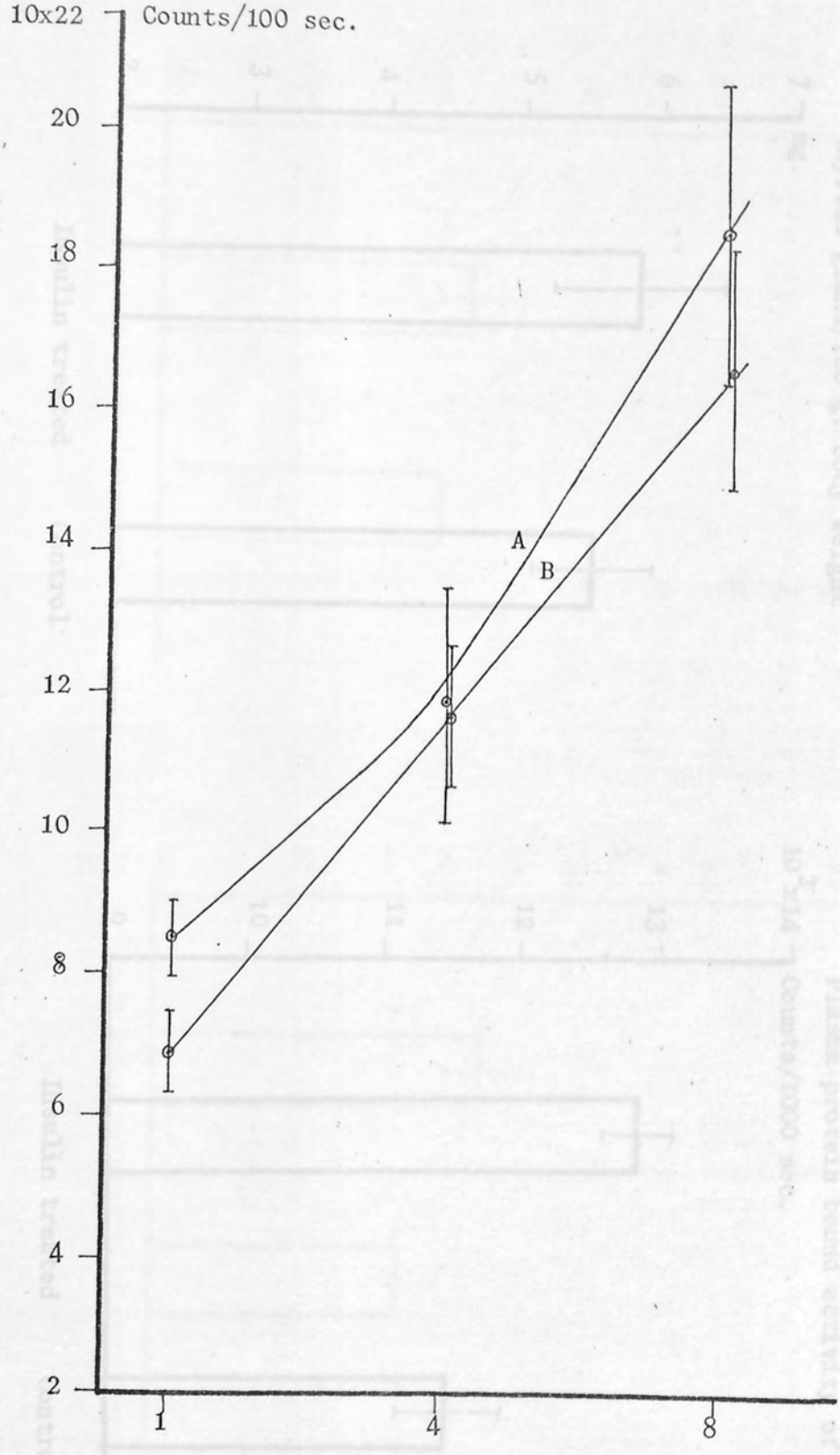


Fig.29

Thyroid gland/100 g. body weight

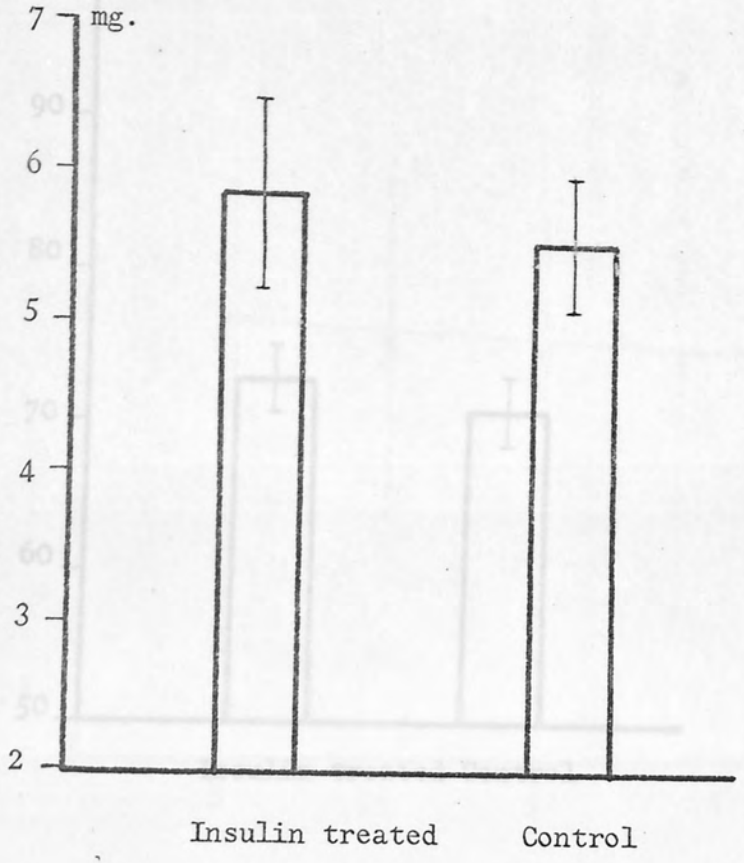


Fig.30

Plasma protein bound activity of ^{131}I

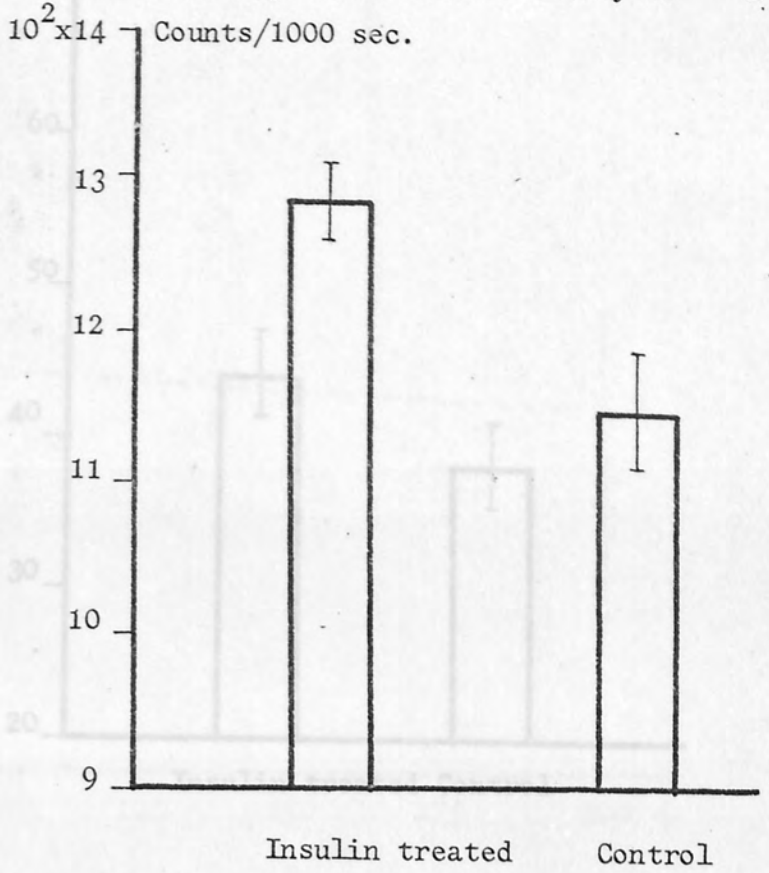


Fig. 31

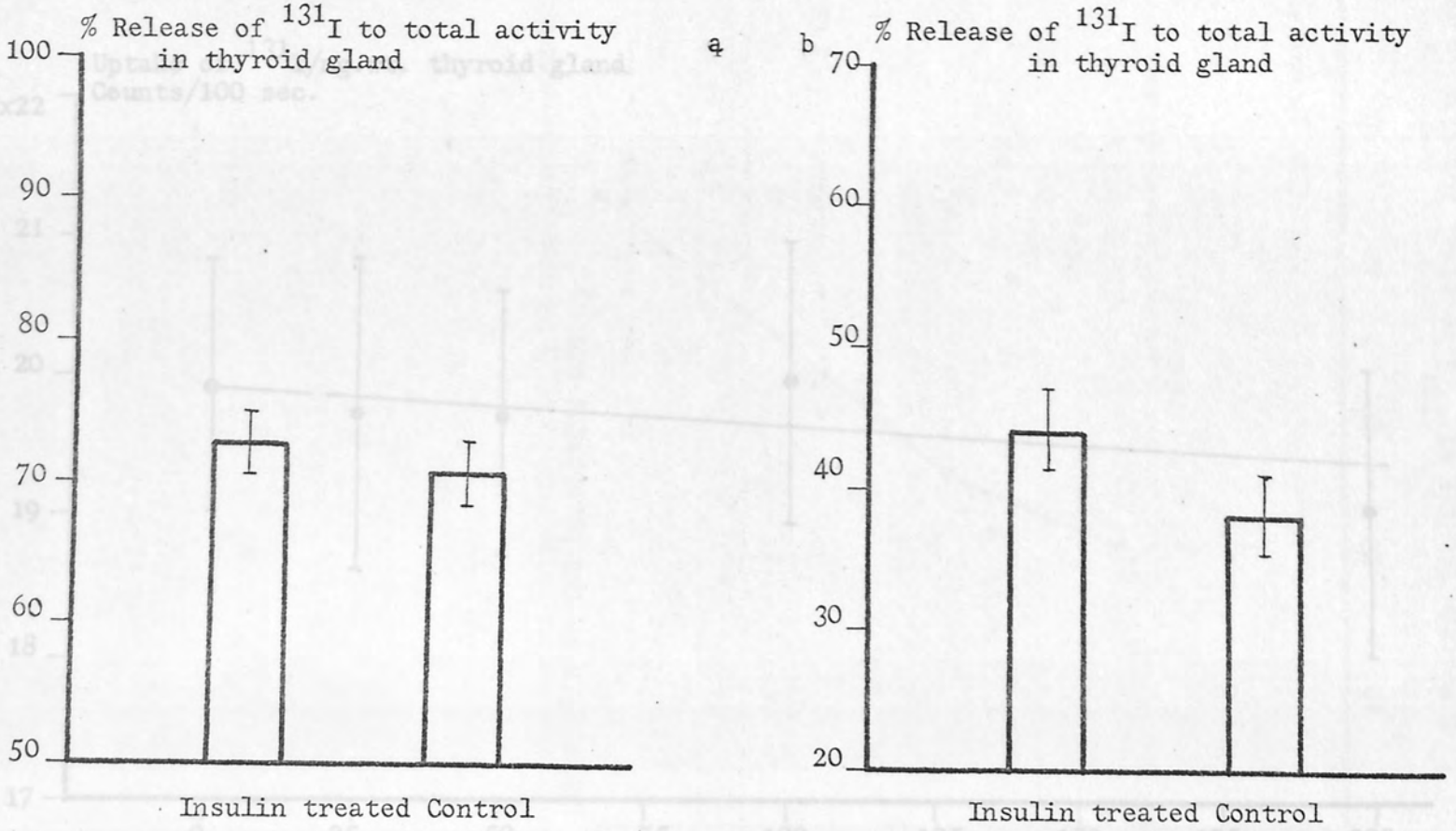

a
% Release of ^{131}I to total activity in thyroid gland
100
90
80
70
60
50
Insulin treated Control
b
% Release of ^{131}I to total activity in thyroid gland
70
60
50
40
30
20
Insulin treated Control

Fig.32.

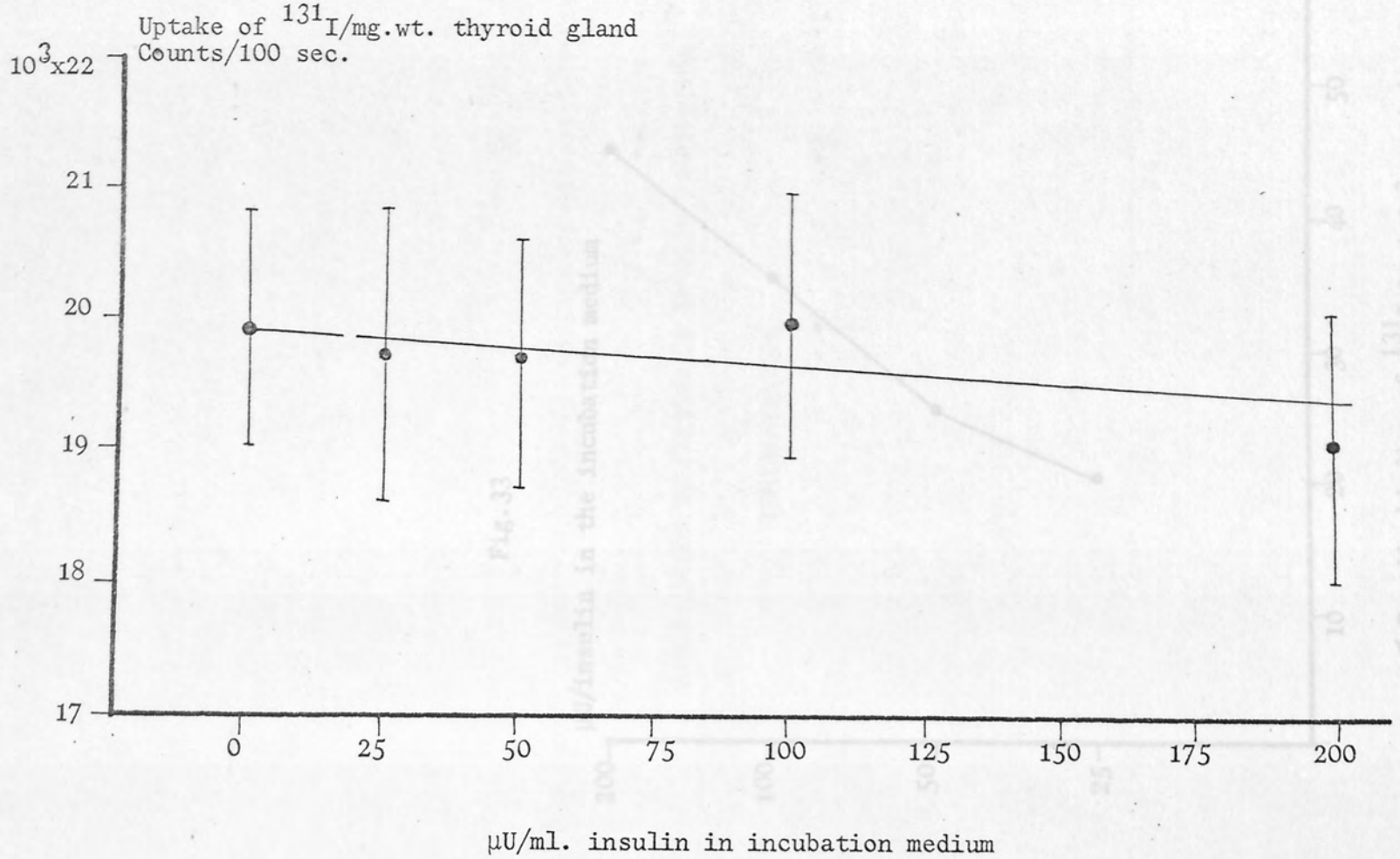
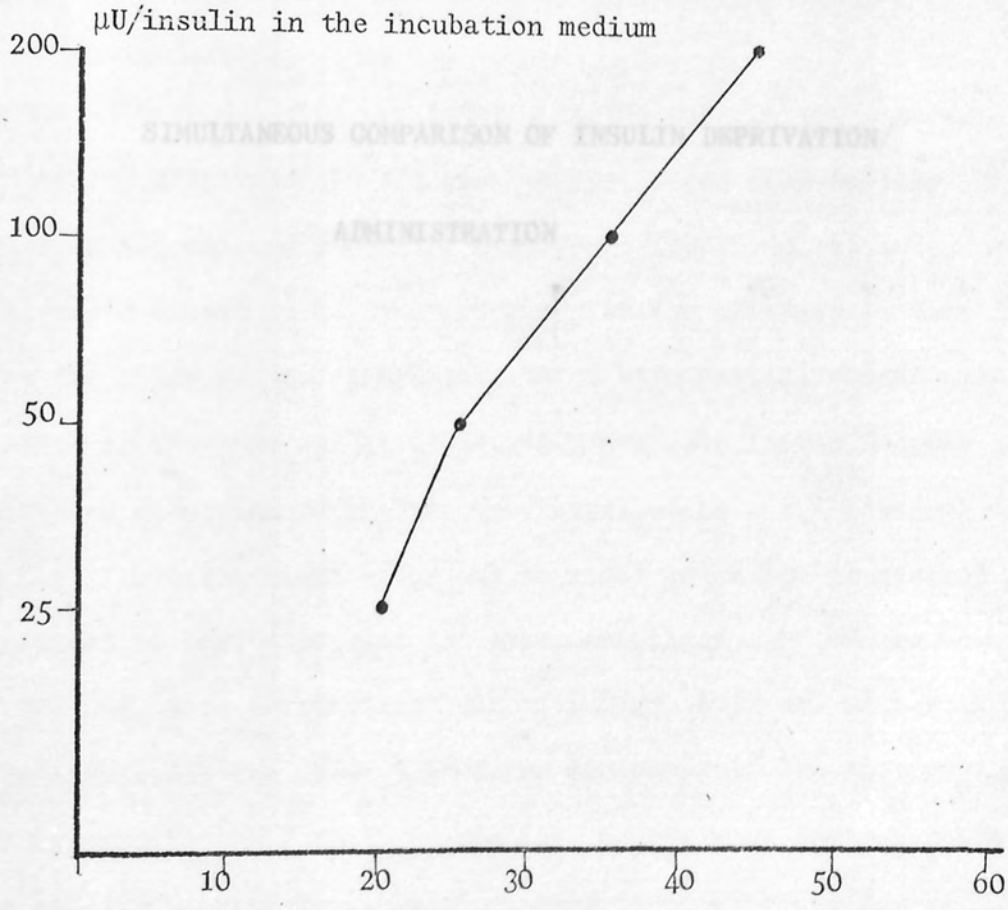

Uptake of ^{131}I/mg.wt. thyroid gland
Counts/100 sec.
10^3x22
21
20
19
18
17
0
25
50
75
100
125
150
175
200
µU/ml. insulin in incubation medium

Fig.33


µU/insulin in the incubation medium
200
100
50
25
10
20
30
40
50
60
% Protein binding of 131I to total activity in thyroid

SIMULTANEOUS COMPARISON OF INSULIN DEPRIVATION/

ADMINISTRATION

INTRODUCTION

In both the previous chapters, the experiments on the thyroid gland activity were performed independently with their own control. The doses of the radio-iodine administered were different and the uptake of radio-iodine was measured at different intervals. It seems difficult to carry out a comparative study of both the induced states simultaneously. The experiment here described is an attempt to observe the thyroid gland activity simultaneously under both conditions of insulin deprivation and excess administration with the same control.

MATERIALS AND METHODS

The alloxan diabetes was induced in the rats in the same way as described previously in the section 'prolonged diabetes' in the chapter on 'Diminished endocrine pancreatic functional state'. Six animals were caused to be severely diabetic for at least 15 days before the start of the experiment. Rats were partially pancreatectomised in the same way as described previously in the Chapter on 'Diminished endocrine pancreatic functional state'. A total of 6 rats kept for seven weeks after the surgical operation of partial pancreatectomy were also used for this experiment.

Six rats were administered with 1.I.U/kg. body wt. of insulin for 20 days under the same procedures described in the section 'Many doses of insulin' on Page 41. Finally, 6 rats were kept untreated under similar housing conditions and considered as controls.

Each rat was administered with 5 μci of ^{131}I ($Na^{131}I$). The uptake of radio-iodine was determined one, four and eight hours after the radio-iodine injection. Forty-eight hours after the

radio-iodine injection, the animals were killed in batches of four; one each from alloxan diabetics, partially pancreatectomised, exogenous insulin treated and untreated or control, by a blow on the head. Five ml. of venous blood was taken out for the estimation of plasma protein bound ^{131}I. The thyroid gland was excised out, cleaned of fatty tissue and weighed on a sensitive balance.

RESULTS

In general the results confirmed the results of the experiments of the same nature described previously.

Fig.34 shows the uptake of ^{131}I calculated as counts/mg. wt. of the thyroid gland. The uptake was least in the alloxan diabetics, followed by partial pancreatectomy, where the uptake of radio-iodine was lower at the first and eighth hour but failed to show any difference at fourth hour uptake. In the insulin treated rats the uptake of radio-iodine was significantly higher at the first hour, but failed to show any difference at the fourth and eighth hour.

In Fig.35 the average of the weights of the thyroid gland of the various groups per hundred gms. of the body weight are shown. Partial pancreatectomised and insulin treated rats failed to show any difference, while in the alloxan diabetics the weight significantly increased over the weight of the control. ($P < .05$).

In Fig.36, the plasma protein bound ^{131}I ($PB^{131}I$) levels are shown. In the alloxan diabetics and in the partial pancreatectomised the $PB^{131}I$ was found to be significantly decreased. The $PB^{131}I$ in the alloxan diabetics was 28% and in the partially pancreatectomised 73% of the control rats. $PB^{131}I$ significantly increased in the insulin treated, where it was 11% more than the control rats. ($P < .001$,

$P > .05$, and $P < .05$ for alloxan diabetics, partially pancreatectomised and insulin treated respectively.)

DISCUSSION

The results and particularly those of the plasma protein bound ^{131}I of all the groups comparatively but quite comprehensively illustrate the role of the deprivation and the result of exogenous administration of insulin in thyroid gland activity. In partial pancreatectomy the partial deprivation of insulin led to decreased iodine hormone release rate. Somehow or other similar deprivation in alloxan diabetes resulted in far less decrease, comparatively, in the release rate of iodine. This shows that the presence of another factor like "hyperketonaemia" must be promoting the action of the inhibition on the release rate, but the other factor looks to be acting differently as it resulted in the increase of thyroid weight like a goitrogen. The action like a goitrogen indicates the involvement of the pituitary and possibly of the hypothalamus, through which the alterations in the thyroid gland activity are mediated under any functional state.

The exogenous insulin had shown antagonistic effect to the deprivation, i.e. stimulation in the release of iodine hormone. The production of the surplus iodine (hormone) under the influence of surplus of insulin shows that the hormone insulin under normal conditions is vital for the normal functioning of the release of ^{131}I and other thyroid gland activity. However, to relate its effect through the pituitary or hypothalamus here seems quite difficult.

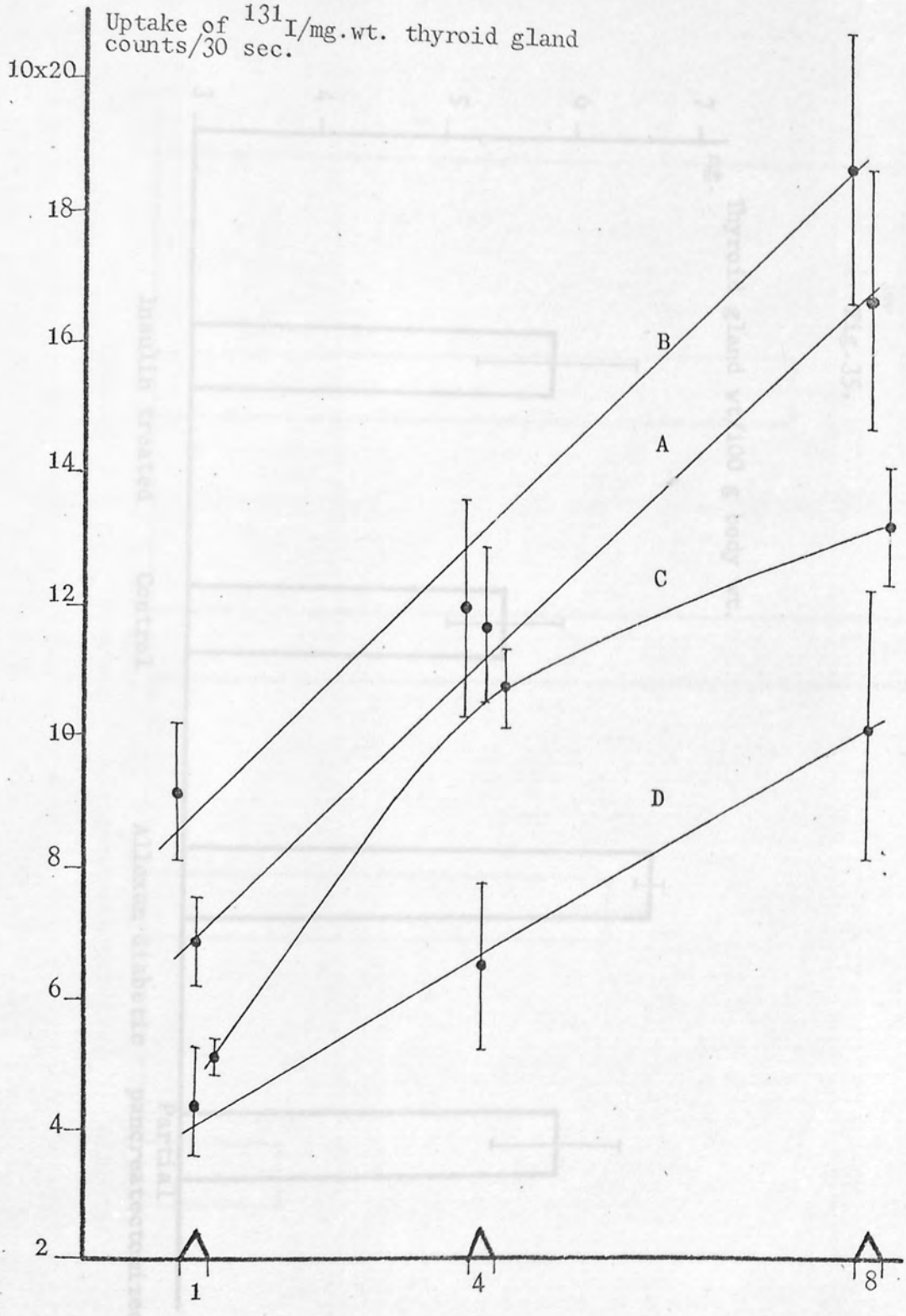
Fig.34. Uptake of ^{131}I expressed as counts/mg.wt. thyroid gland in control, insulin treated, partial pancreatectomized and alloxan diabetic rats.

A Control
B Insulin treated
C Partial pancreatectomized
D Alloxan diabetic

Fig.35. The average weights of thyroid gland/100 g body weight in control insulin treated, partial pancreatectomized and alloxan diabetic rats.

Fig.36. Plasma protein bound ^{131}I in control, insulin treated, partial pancreatectomized and alloxan diabetic rats.

Fig. 34.



Hours after first injection

Fig.35.

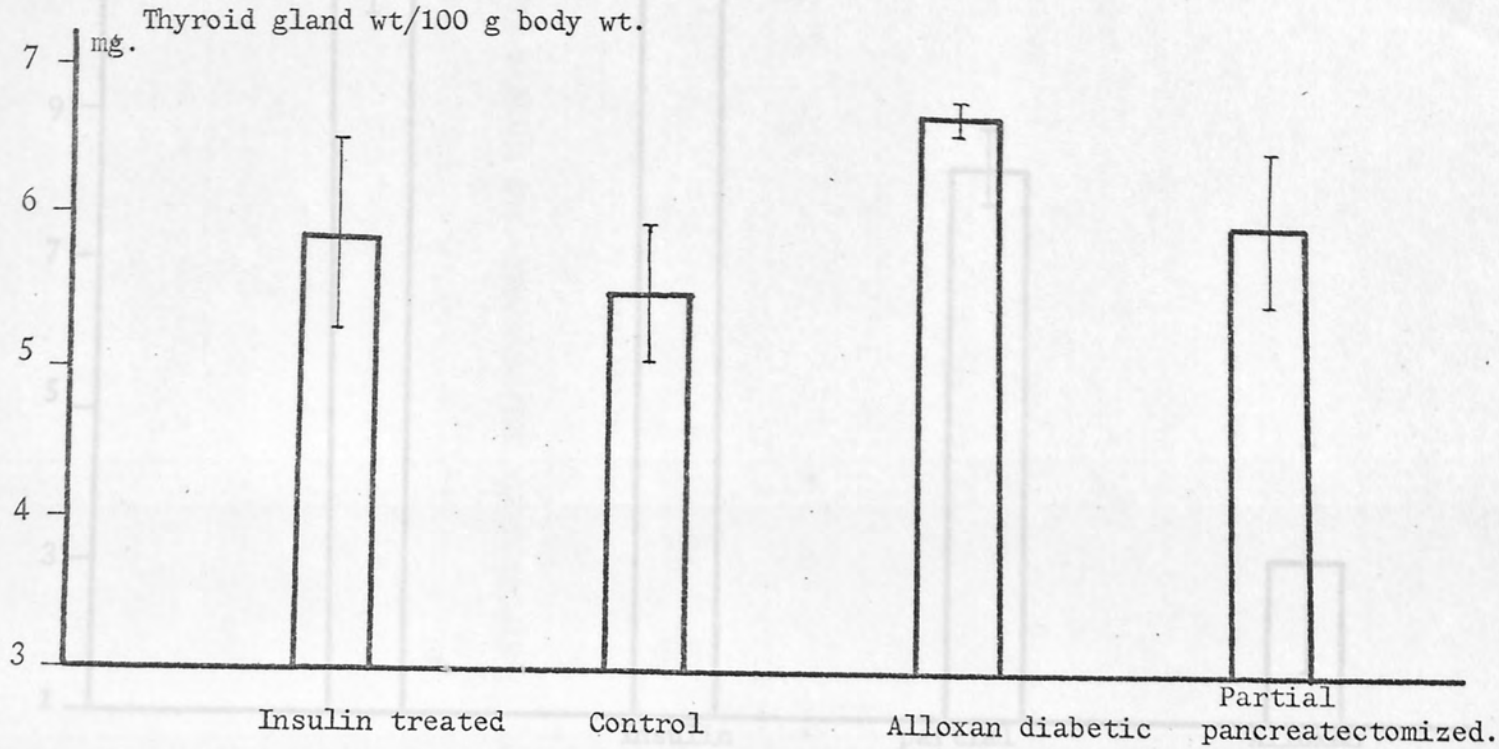
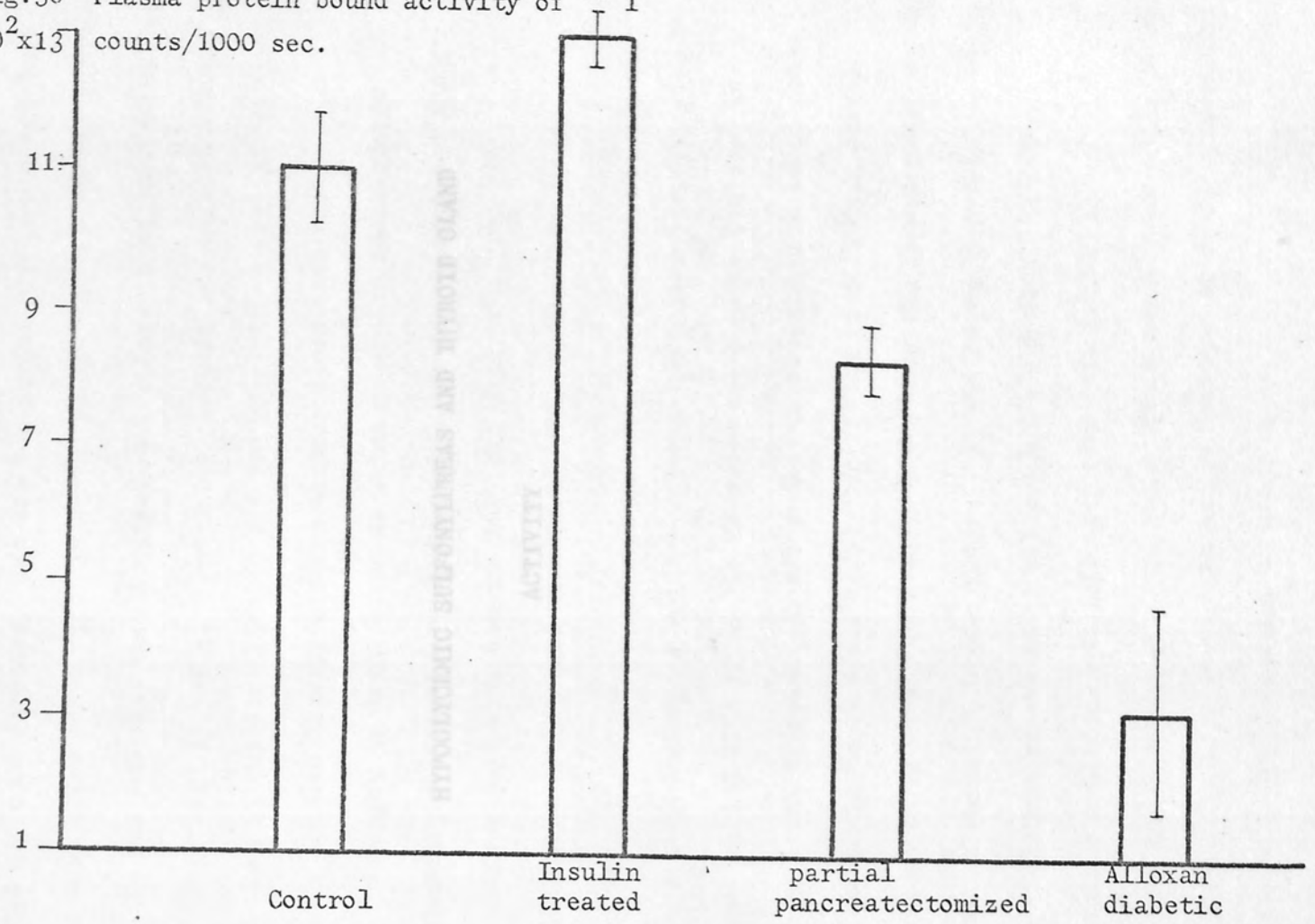

Thyroid gland wt/100 g body wt.
mg.
7
6
5
4
3
Insulin treated
Control
Alloxan diabetic
Partial
pancreatectomized.

Fig.36 Plasma protein bound activity of ^{131}I



HYPOGLYCEMIC SULFONYLUREAS AND THYROID GLAND ACTIVITY

INTRODUCTION

It was immediately apparent after the discovery of insulin that it did not cure diabetes, that its beneficial effect did not last more than a few hours, that injection needed to be repeated throughout the day and that such treatment would have to be carried out indefinitely. It was also shown that insulin was rapidly inactivated when it was administered via the digestive tract and in certain aspects insulin therapy was not without disadvantages.

Attempts to treat human diabetes by orally administered pharmacological agents and in particular by synthalins and their derivatives were made between 1925 and 1930 (186,187, 188). These substances were absorbed because of their variability concerning their mode of action.

A sulphonamide, p-amino-benzene-sulphonamide-isopropyl-thiodiazol (called 2254 RP by the French Rhone-Poulenc Society) was used to treat typhoid. In all patients treated with large doses of 2254 RP up to 12 g/day (189) three patients died from obscure causes which were only elucidated later when the hypoglycemic action of the sulphonamide became clear. It was strongly suggested that they died from severe and prolonged hypoglycemia. In other patients convulsions and prolonged coma were noted; in a few patients the lowering of blood sugar levels was also causally noted. Administration of glucose, whether by accident or design, succeeded in reversing these symptoms in a few cases.

Later it was seen that oral administration of a single dose of 2254 RP (0.25 g./kg. body wt.) caused in normal, conscious, fasting dogs, a progressive hypoglycemic both prolonged and severe

which lowered the blood sugar levels; 24 hours after the administration of the compound, blood sugar levels had not yet returned to normal (190, 191). It was suggested that this sulphonamide is a substance which shows 'Pancreatic tropism'. It acts on the endocrine system of the cells of the islets of Langerhans, stimulating them to liberate increased quantities of endogenous insulin into the blood and this endogenous insulin liberated in excess is responsible for the hypoglycemic effect (190).

German workers published results which they had obtained with a sulphonamide, 1-butyl-3-sulfonylurea or carbutamide (192,193,194) on which the main study of the hypoglycemic sulphonamides in Germany was based.

Systematic treatment of diabetes with hypoglycemic sulphonamide was not instituted until 1955 because of the possible toxic effects and uncertainty concerning the action of these compounds. Significant hypoglycemia was then reported in diabetics with one of these active sulphonamides and it was then found that diabetics could be satisfactorily treated with this substance carbutamide (BZ 55) (195).

A large number of similar compounds of the sulfonylurea group were found to be suitable for the treatment of diabetes. From this time onward research in the field of the oral-antidiabetic agents blossomed out and was conducted in a most extensive and widespread manner in laboratories all over the world.

Of the sulfonylureas, carbutamide and tolbutamide, by virtue of their therapeutic excellence have been the standard therapy for more than a decade for the treatment of diabetes.

There is unequivocal evidence that the sulfonylureas stimulate

the beta cells of the islets and liberate into the blood an accumulated quantity of endogenous insulin, as they fail to induce a hypoglycemic effect in the completely pancreatectomized dog. (196, 197, 198), rat (199, 200), cat (201) and toad (202). Similarly severely alloxan diabetic dogs (196,203,204,205), rabbits (190, 203, 206, 207, 208, 209) and rats (193,210,211) have no hypoglycemic response to the sulfonylureas. However, in partially or incompletely alloxanized animals having mild glycosuria and hyperglycemia but no ketonemia, these drugs do cause reduction in blood glucose concentration (192,198,212,213). Very shortly after the introduction of the sulfonylurea it was suspected that their hypoglycemic action might be due to cytotoxic effect on α cells, with reduction or suppression of glucagon secretion (192,193,194). Experiments with synthalin, 2254 RP (214,215) and the earlier histological studies in the animals given carbutamide (216,217) or tolbutamide (218) seemed to support this hypothesis. However, attempts to repeat or confirm these observations have been unsuccessful. Thus studies in rats (219,220,221), guinea pigs (222,223), rabbits (224), dogs (225) and calves (226) have all failed to show significant changes in the alpha cells on short or long term administrations of the sulphonylureas.

Exogenous administration of insulin had proved to play a role in the thyroid gland activity at least in the rat. The sulfonylureas stimulate the release of insulin reserves present in a readily available soluble form. An attempt has been made in this work to study thyroid gland activity with enhanced levels of endogenous insulin induced hy sulfonylureas.

The duration and amount of blood sugar reduction by sulfonylurea

is related within certain limits to the plasma concentrations of the drug. The relationship between blood sulfonylurea concentration and hypoglycemic response has been studied by many, both for carbutamide (192,227,228) and tolbutamide (229,230,231), the hypoglycemic response increasing as the concentration reaches 10 - 20 mg%. A significant relationship between carbutamide dose and fall in blood glucose level was found for doses given intravenously to rabbits between 0.05 and 0.25 g/kg. body wt. and for doses of 0.05 - 0.15 g/kg. body wt. given orally to rats (192, 227, 231,232). Smaller doses had no hypoglycemic effects. With tolbutamide the relationship of dose to response is valid over only a limited range, corresponding relationship for the various animals and method of administration of tolbutamide are as follows (196,229, 230,231,233), dog, 50 - 100 mg/kg. orally; rabbit 50 - 500 mg/kg. orally and 6 - 72 mg/kg. h. in intravenous drip; rat, 100 - 250 mg/kg. orally and mouse 250 - 500 mg/kg. subcutaneously.

Tolbutamide does not cause any significant changes in ^{131}I tests of thyroid function, in the serum protein bound ^{131}I or in basal metabolic rate (234,235,236,237). This also applies to carbutamide, provided very large doses are not given. Only minor "hypothyroid-like" changes were seen in the patients given carbutamide 2.0 g daily for nine weeks (235), these changes were abolished by withdrawal of the drug. Serum cholesterol, protein bound iodine levels and thyroid iodine uptake and secretion were studied in 116 patients treated with carbutamide for 1 - 3 years (238), the results of these did not differ from those in untreated controls but a few days after stopping sulfonylureas a significant increase in ^{131}I uptake was noted. A few cases of goitre (239) or hypothyroidism

(240) during carbutamide therapy were reported but the association is probably fortuitious. There is no impairment of the hypoglycemic response of the sulfonylureas in patients with thyrotoxicosis or myxodema.

Large doses of tolbutamide or carbutamide for long periods caused enlargement of and morphological changes in the thyroid of rats and dogs (192,237, 241) and an increase in plasma cholesterol (242). These effects are similar to those of mild thiouracil-type goitrogenic drugs (237). Carbutamide showed a considerably greater effect on the thyroid than did tolbutamide which did not increase the thyroid weight after 4 weeks (243) or alter thyroid function as reflected by ^{131}I tests and plasma protein bound iodine content (237).

The introduction of carbutamide showed changes which were consistent with increased glandular function and decreased hormone secretion (192,244). After prolonged treatment with carbutamide some experimental animals were found to have thyroid glands weighing up to four times their normal weight (245,246,247,248). In some cases localized as well as diffused hyperplasia was observed. Histological changes seen were colloid depletion, enlargement of the epithelium with swelling of the nuclei and hyperaemia. The iodine content and uptake of the gland were decreased. Some animals given carbutamide for very long periods were found to have "thyroidectomy cells" (i.e. polygonal and vacuolized beta cells in the anterior pituitary). Tolbutamide has a similar but considerably less marked effect on the thyroid (237).

In man carbutamide has been shown to reduce thyroid uptake of ^{131}I and lower the basal metabolic rate and the protein bound iodine level. These effects were only temporary and normal values were found on discontinuation of the therapy (233). With tolbutamide

the effects were much less or absent. Some workers observed a decrease in P.B.I. and ^{131}I uptake in subjects taking chloropropamide, but no clinical evidence of hypothyroidism (248, 249). Stratmann (239) described three diabetics with goitre in whom the gland increased markedly in size while on tolbutamide. Crentzfeldt and Soling (195) quote two cases in which myxodema occurred, one with carbutamide and one with tolbutamide and subsided after discontinuation of the drug. Nikkila (250) in a carefully controlled study, found no excess of hypothyroidism or goitre in subjects taking carbutamide or tolbutamide although he did observe a rebound increase in ^{131}I uptake after withdrawal of the sulfonylureas. It has been suggested that clinical hypothyroidism is occurring more frequently than would be expected in diabetics on long term treatment with sulfonylureas. Hunton and co-workers (251) found 30 cases of hypothyroidism among 220 patients taking chloropropamide or tolbutamide as against 8 among 229 diabetics treated by other means. They suggest that some 20% of diabetics may be expected to develop hypothyroidism after five years of sulfonylurea therapy. They found no incidence of goitre.

Carbutamide has been shown to contain the amino-benzene structure. It has the amino group in the para position on the aromatic ring, as do sulfadiazine and other amino benzene derivatives, which have been shown by Astwood et al.(252) to have antithyroid activity; otherwise analogous compounds lacking this aromatic amino group had no antithyroid activity (253).

It has been shown in the above literature that large doses of sulfonylureas like carbutamide, tolbutamide and chloropropamide are effective to induce considerable hypoglycemia and antithyroid activity in different animals and man. The increase in the levels

of insulin due to stimulated release of endogenous insulin, even if it creates any thyroid stimulating effect is overcome by a dominant antithyroid effect of the large doses of the sulfonylureas.

One of the newest sulfonylureas to be developed is glibenclamide (HB 419). It was discovered that the addition of an acylaminoalkyl group in the 4th position of the benzol ring of sulfonylurea potentiates its blood sugar lowering effect. On this basis this substance is much stronger than any other antidiabetic agent of the sulphonamide group and has no demonstrable side effects. Thus, the minimal effective oral dose, when compared to tolbutamide, was actually reduced by a factor of 1000 in rabbits and by a factor of 250 in dogs. These results are confirmed by Schwarz et al. and Loubatieres et al. (254). Glibenclamide was found to be 100 times stronger in rats and 1000 times stronger in rabbits than tolbutamide.

In the light of the previous review of the effect of most sulfonylureas like carbutamide, tolbutamide and chloropropamide on the thyroid gland activity, it could be suggested that glibenclamide, being highly hypoglycemic potent and effective in comparison to others should not interfere with the thyroid gland activity.

The aim of the work described here is to observe the effect of the increased release of endogenous insulin on thyroid gland activity, the endogenous insulin secretion being enhanced by administration of glibenclamide.

MATERIALS AND METHODS.

Tolbutamide.

Eight rats weighing between 220 - 250 gms. were used. Each rat was injected with 10 µci of $^{131}I(Na^{131}I)$ and the rate of the release of radioiodine from the thyroid gland was determined. The neck region of the thyroid gland was counted for six times in the

control period. After the sixth, seventh, eighth and ninth counting 4 ml/kg. of body wt. of 'Rastinon' (Tolbutamide as sodium salt) was injected intraperitoneally to each rat. The neck region of the thyroid gland was counted. The neck region of the thyroid was counted for a further eight times after the control period.

The blood samples of rat from the tail region 24 hours before the first injection, three hours after the first and third injections and twenty-four hours after the last 'Rastinon' injection were taken and blood sugar levels were estimated.

Glibenclamide.

Preparation:

500 mg. of glibenclamide was dissolved in a minimum quantity of N/10 NaOH and made up to 25 ml. with deionized water. The pH of the solution was made up to nearly 8.4 with the addition of N/50 HCl and further made up to 50 ml. with deionized water. This was kept as a stock solution. It was sterilised by passing through a millipore filter.

For in vivo studies the stock solution was mixed with deionized water to provide the required concentration.

For in vitro studies the stock solution was mixed with basal medium to provide a required concentration.

In vivo Short-term administration.

Seven rats weighing between 220 - 250 gms. were used. Each rat was injected with 10 µci of $^{131}I(Na^{131}I)$. The rate of the release of radioiodine from the thyroid gland was determined. The neck region of the thyroid gland was counted for six times in the control period. Immediately after the sixth, seventh, eighth and ninth counting 4 mg/kg of body wt. of the prepared glibenclamide

was administered intraperitoneally to each rat. The counting of the neck region of the thyroid gland was carried out for a further eight times after the control period.

The blood samples for estimation of the blood sugar levels were taken 24 hours before the first injection, three hours after the first and third injection and 10 and 34 hours after the last injection.

In vivo long-term administration.

Ten of the twenty rats weighing between 180 - 220 gms. were used and kept on drinking water containing 0.001% of glibenclamide for 40 days. The other ten were kept on normal water and considered as controls. Each rat was administered with 10 µci of $^{131}I(Na^{131}I)$. After the end of the period of the glibenclamide administration in the experimental group the uptake of radioiodine by the thyroid gland was determined at the 1st., 3rd., 6th, 20th and 43rd. hour. 48 hours after the radioiodine injection, the animals were killed with a blow on their head and 5 ml. of venous blood was taken out and was used for the estimation of plasma protein bound ^{131}I. Thyroid glands were excised out, cleaned of fatty tissue and weighed carefully in a sensitive balance. The thyroid glands were homogenized and treated for the estimation of protein bound ^{131}I in the thyroid gland.

in vitro Secretory rate

Twenty-nine mice of T-O strain were kept on a low iodine diet for a week and injected with 0.2 µci of $^{131}I(Na^{131}I)$. After six hours the animals were killed with a blow on the head. The thyroid glands of the animal were excised out and incubated for the study of the release of ^{131}I. The experimental dishes contained 20 µg/ml. of the prepared glibenclamide in the incubation medium.

2.0 μ mole of sodium perchlorate were added to the dishes of both experimental and control thyroids to block the recapture of ^{131}I by the thyroid. The thyroids were incubated for 48 hours. The activity of ^{131}I left in the thyroid gland and released in the incubation medium was determined.

in vitro Uptake and protein binding.

A concentration of 20 μg/ml. of the prepared glibenclamide in the incubation medium was used for ten excised thyroids of the T-O strain mice. Ten other thyroids were cultured in the normal incubation medium and considered as controls.

At first all the experimental and control thyroids were cultured in a normal medium for six hours; then transferred to the fresh medium, the experimentals with prepared glibenclamide and the controls without it. These were incubated for 12 hours and 0.2 μci of ^{131}I($Na^{131}I$) was added to each dish. The incubation was carried on for a further 6 hours.

Uptake of ^{131}I in the thyroid gland was measured, the thyroids were weighed carefully in a sensitive balance and were treated with trichloroacetic acid to estimate the protein bound ^{131}I.

RESULTS

Tolbutamide.

Fig.37 shows the release of ^{131}I from the thyroid gland throughout the experiment. Quite regular release of radioiodine was observed in the control period. The release rate remained unaltered for nearly 24 hours after the first injection of 'Rastinon'. However, a gradual inhibition in the release rate was observed afterwards. The effect remained persistent long after the withdrawal of the drug.

As shown in Fig.38 the blood sugar levels were quite normal in the control period and considerable hypoglycemia was induced after the 'Rastinon' administration. The hypoglycemic effect seemed to persist long after the injection of 'Rastinon', but gradually returned to normal.

Glibenclamide

in vivo Short-term administration

Fig.39 shows the release rate of ^{131}I from the thyroid gland throughout the experimental period. Quite a regular release was observed through the control period. The treatment and after treatment periods showed that glibenclamide did not affect the release rate of the radioiodine.

As shown in Fig.40 the blood sugar level was quite normal in the control period and considerable hypoglycemia was induced after the administration of glibenclamide. The hypoglycemic effect seemed to persist long after the injection, but gradually returned to normal.

in vivo Long-term administration

Fig.41 shows the uptake of ^{131}I calculated as counts/mg. wet wt. of thyroid gland. The uptake of ^{131}I was significantly lower in the glibenclamide treated rats than in the controls. The counts of ^{131}I at the 6th, 20th and 43rd. hour showed that the curve of release rate at these early hours was inhibited in the glibenclamide treated rats more than the control.

In Fig.42 the average wet wt. of the thyroid gland per 100 gm. of the body wt. of the rats is shown. The wt. of the glibenclamide treated rats significantly increased over the control rats ($P < .001$).

In Fig.43 the average percentage protein bound ^{131}I in the thyroid gland has been shown. The percentage of protein bound ^{131}I significantly decreased in the glibenclamide administered rats. ($P < .01$).

In Fig.44 the average of the plasma protein bound ^{131}I (PB ^{131}I) level is shown. The level in the glibenclamide treated rats was significantly lower than in the controls ($P < .01$).

in vitro Secretory rate

Fig.45 shows the secretory rate of ^{131}I from the incubated thyroid gland. Glibenclamide failed to show any effect on the release rate of radio-iodine from the control rats.

in vitro Uptake and protein binding

Fig.46 shows the uptake of ^{131}I calculated as counts/mg. wet wt. of the thyroid gland. Glibenclamide failed to show any effect on the uptake of ^{131}I.

Fig.47 shows the percentage protein bound ^{131}I in the thyroid gland. The percentage protein binding of ^{131}I in the thyroid gland incubated in the glibenclamide added medium was significantly lower than in the thyroids in the control medium.

DISCUSSION.

In the experiments with tolbutamide and glibenclamide, both have been shown to induce significant hypoglycemia, the direct result of beta cell stimulation enhancing the levels of the endogenous insulin in the blood of the rats. Tolbutamide in the dose, generally employed for obtaining a considerable hypoglycemia, proved to be anti-thyroid as shown in the experiments of this work and by many other workers.

It seems quite impossible to discuss the effect of the induced excess of endogenous insulin on the thyroid gland activity under the

interfering factor of anti-thyroid effect of tolbutamide. It could be postulated that excess of endogenous insulin induced with tolbutamide might have been affecting or stimulating the thyroid gland activity as with exogenous administered insulin on one side and tolbutamide inhibiting the thyroid gland activity on the other side; but the levels of tolbutamide in the blood inhibiting thyroid gland activity show more potency over the levels of insulin stimulating thyroid gland activity; thus the sum of the two activities leads to an apparent anti-thyroid influence of tolbutamide in the animals.

It was thought that an experiment to block the stimulating effect of the sulfonylureas and to compare the thyroid gland activity under such conditions with that of unblocked controls might be performed. The increase in the antithyroid activity of the blocked animal would be due to the absence of excess of endogenous insulin in comparison to unblocked controls where the excess of endogenous insulin might be stimulating the activity along with the anti-thyroid activity of the sulfonylureas. However, this idea of blockage has to be abandoned for the reason that the blockage chemicals may not show their own effect on the thyroid gland activity and in the case of the blockage of even normal insulin release with the excess of blockage chemicals, it seemed difficult to sum out the effect of excess of endogenous insulin on the thyroid gland activity induced by sulfonylureas.

Glibenclamide did not show any effect on the thyroid gland activity on the <u>in vivo</u> thyroid secretory rate, although significant hypoglycemia was induced by the doses administered. These doses of glibenclamide were 1/500th of the doses of tolbutamide. The possibility of the antithyroid activity of such doses of gliben-

clamide should be negligible as the similar doses of tolbutamide failed to show any effect on the thyroid gland activity (234,235).

However, the results of the in vivo release of radioiodine under the administration of glibenclamide can be discussed under two possibilities:

1. Considering that glibenclamide does not show any antithyroid activity and induces considerable hypoglycemia or excess release of endogenous insulin, the non-effect of glibenclamide in short-term treatment could suggest that excess release of endogenous insulin does not affect the thyroid gland activity. However, this seems improbable as in later experiments glibenclamide did show anti-thyroid activity like other sulfonylureas.

2. The excess of release of endogenous insulin does stimulate the thyroid gland activity, but a simultaneous anti-thyroid activity failed to show the apparent effect of it.

Although both possibilities fail to give any clue to the excess release of endogenous insulin effects on the thyroid gland both possibilities do prove that glibenclamide is an antithyroid agent and much more potent than other sulfonylureas.

It has been shown that in all the sulphonamide compounds, the basic composition of the molecule - SO_2 - NH - C is a pre-requisite for hypoglycemic action (255). The organic compounds investigated for antithyroid potential appear to require the presence of -NH - C(=S) - NH or C_6H_4 - NH groupings for activity(256).

It could be suggested that the molecular configuration in the glibenclamide which is responsible for the increased potency of the hypoglycemic action in comparison to other sulfonylureas may be simultaneously responsible for the increased potency for anti-thyroid

activity.

Thus glibenclamide like the other sulfonylureas proves to be a potential antithyroid compound. In its anti-thyroid effect, it resembles the goitrogens like thiourea, thiouracil but not perchlorate or other inorganic goitrogens. Like thiourea and thiouracil it would appear that glibenclamide allows iodine to enter the thyroid but the iodination of tyrosin in the gland is blocked (257, 258,259). Unlike propylthiouracil, which shows a maximum blockage of thyroid iodine by the third day (237) and significant increase in the thyroid weight by the end of the first day (237), short term treatment with glibenclamide does not show any effect on ^{131}I release in the rat thyrcid.

Fig.37. The release of ^{131}I from the thyroid gland of tolbutamide treated rats.

↓ Injection of 'Rastinon'

Fig.38. Blood sugar level at the different times in the above experiment.

Fig.39. The release of ^{131}I from the thyroid gland of glibenclamide treated rats.

↓ Injection of sodium salt of glibenclamide.

Fig.40. Blood sugar level at the different times in the above experiment.

Fig.41. Uptake of ^{131}I expressed as counts/mg.wt. of thyroid gland in control and glibenclamide treated rats.

A Control

B Glibenclamide treated.

Fig.42. The average weights of thyroid gland/100 g body wt. in control and glibenclamide treated rats.

Fig.43. Protein bound ^{131}I in the thyroid gland of control and glibenclamide treated rats.

Fig.44. Plasma protein bound ^{131}I in control and glibenclamide treated rats.

Fig.45. In vitro release rate of ^{131}I from the thyroid gland of the mouse incubated in control and glibenclamide treated rats.

Fig.46. Uptake of ^{131}I expressed as counts/mg. wt. thyroid gland of the mouse incubated in control and glibenclamide added culture medium.

Fig.47. Protein bound ^{131}I in the thyroid gland of the mouse incubated in control and glibenclamide added culture medium.

Fig. 37

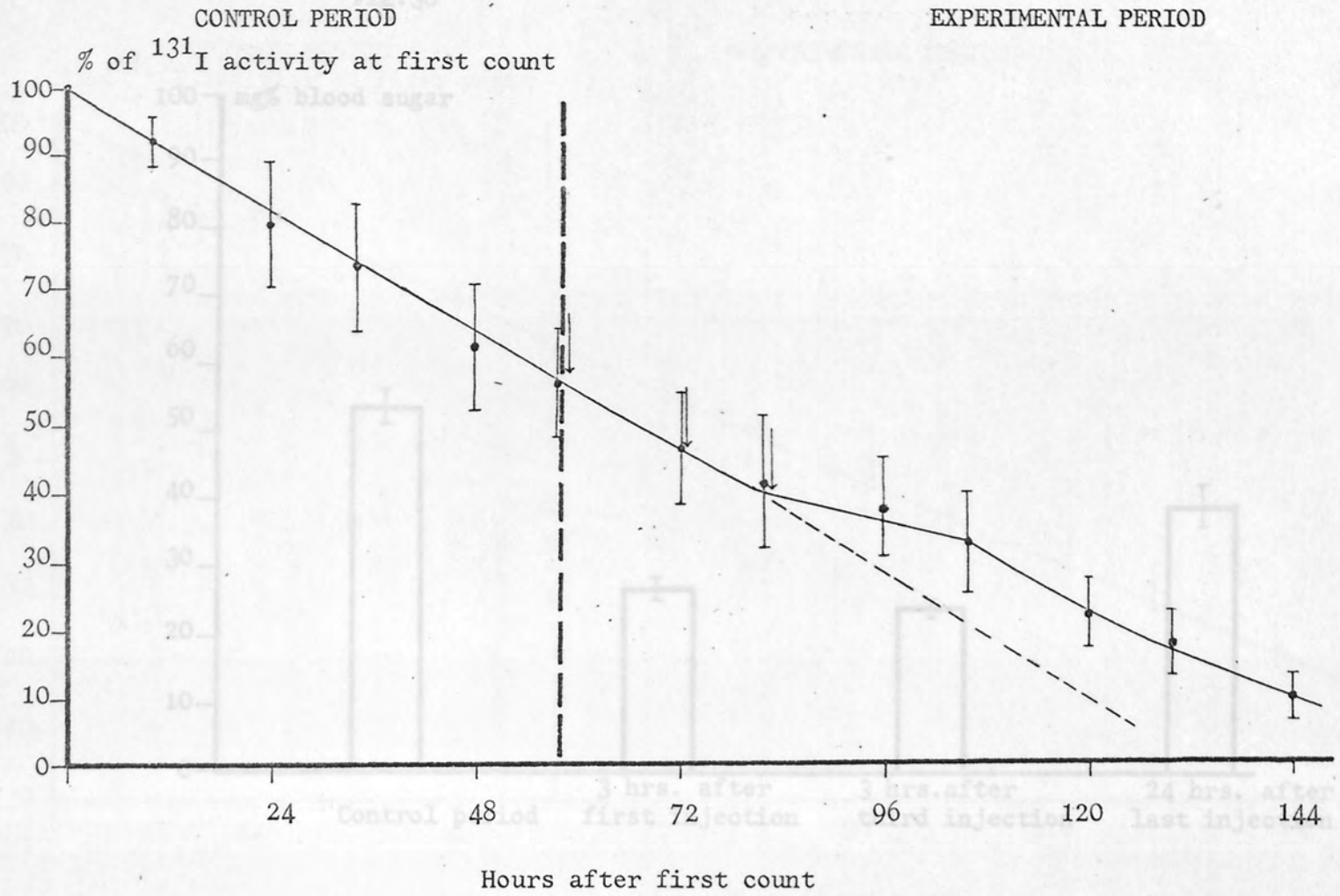

CONTROL PERIOD
EXPERIMENTAL PERIOD
% of 131I activity at first count
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
120
144
Hours after first count

Fig.38

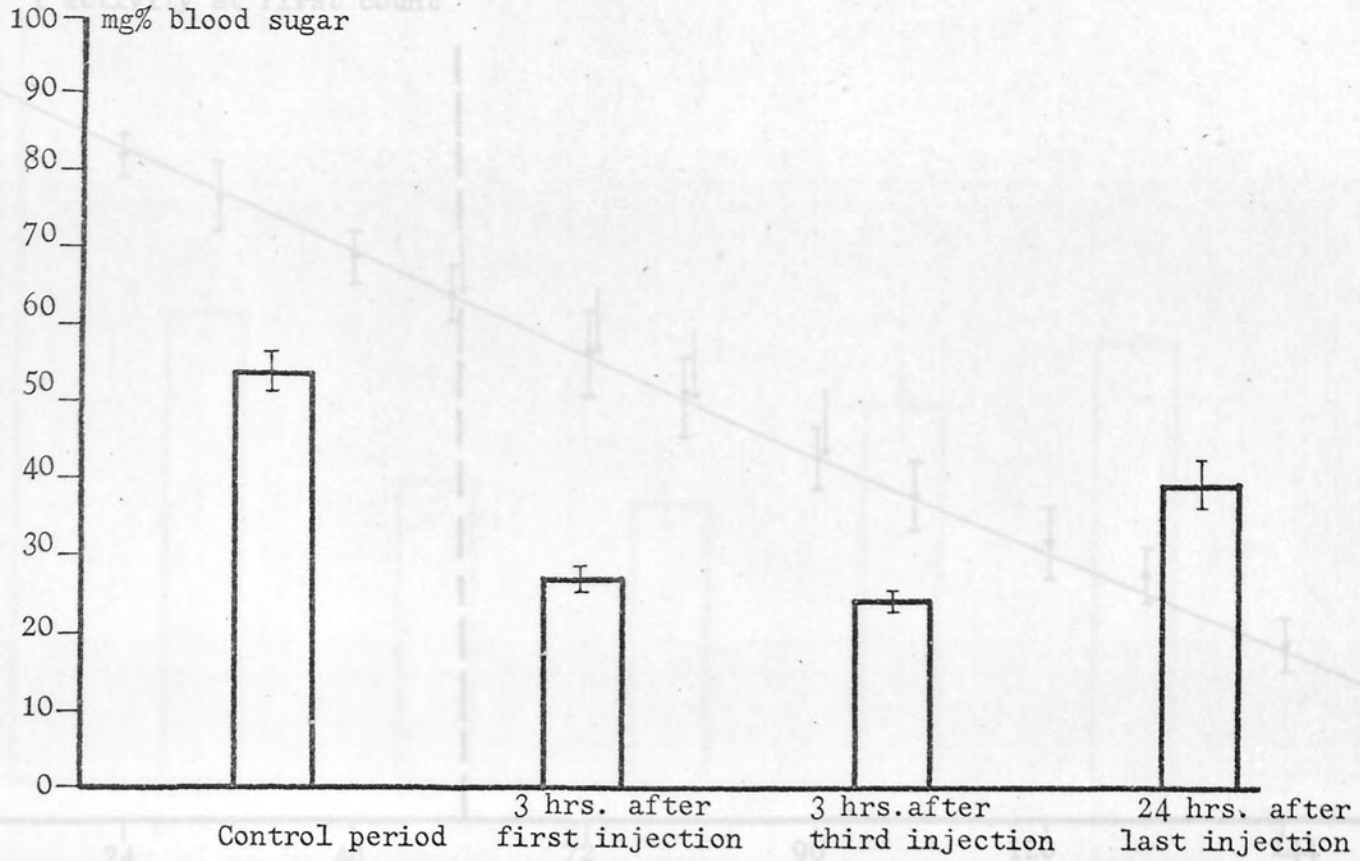

100
mg% blood sugar
90
80
70
60
50
40
30
20
10
0
Control period
3 hrs. after first injection
3 hrs. after third injection
24 hrs. after last injection

Fig.39

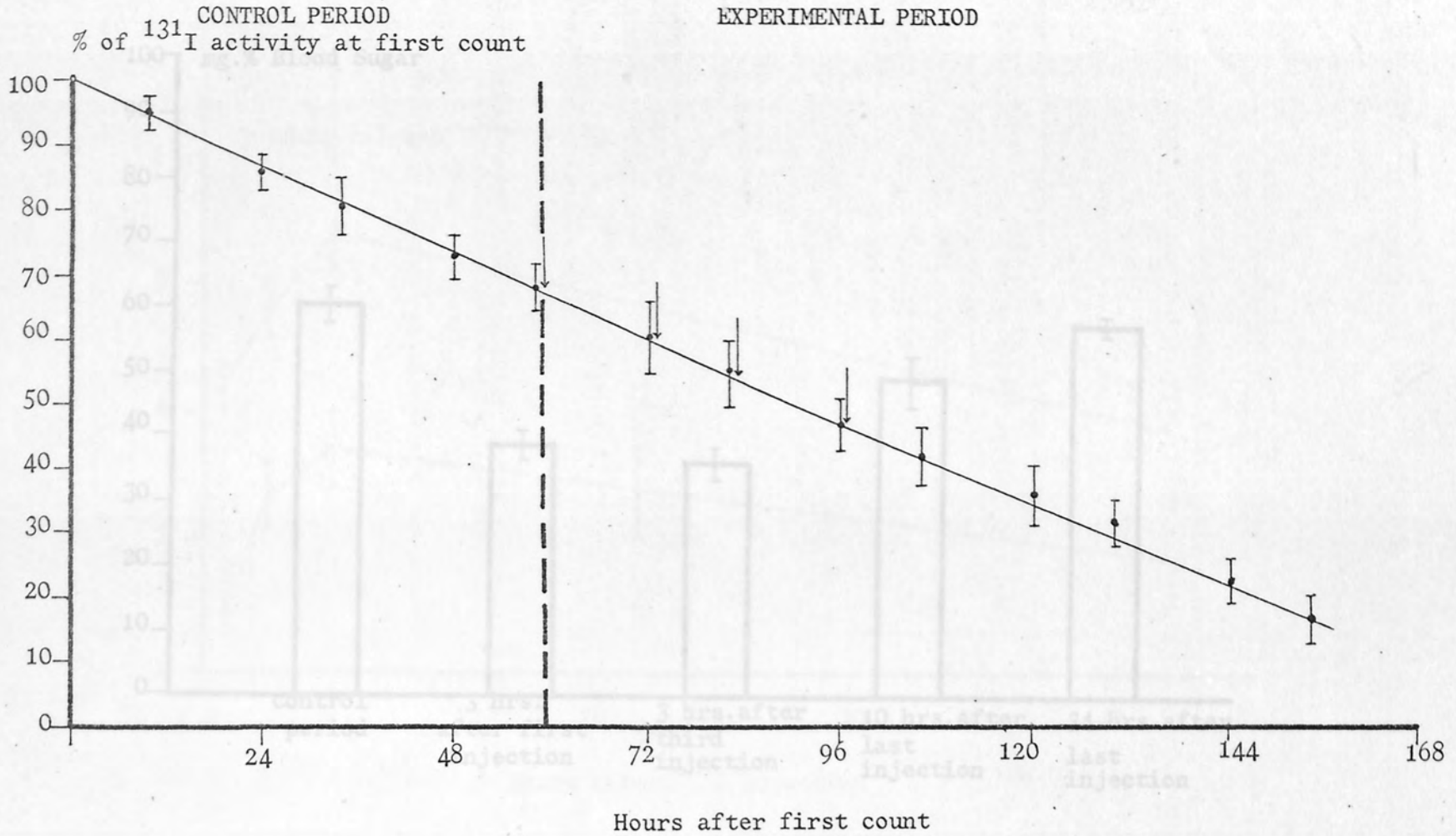

CONTROL PERIOD
EXPERIMENTAL PERIOD
% of 131I activity at first count
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
120
144
168
Hours after first count

Fig.40

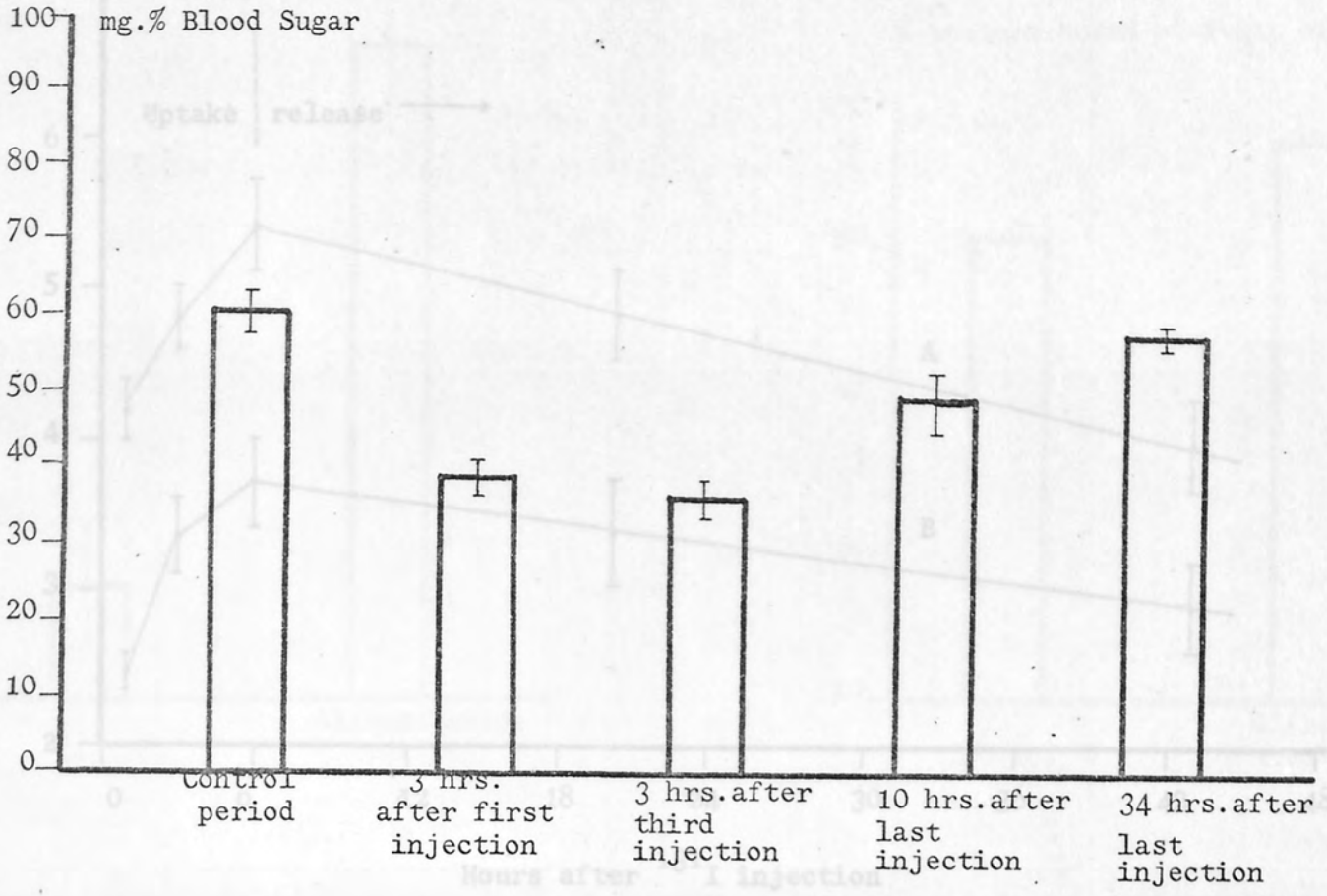

mg.% Blood Sugar
100
90
80
70
60
50
40
30
20
10
0
Control period
3 hrs. after first injection
3 hrs.after third injection
10 hrs.after last injection
34 hrs.after last injection

Fig.41.

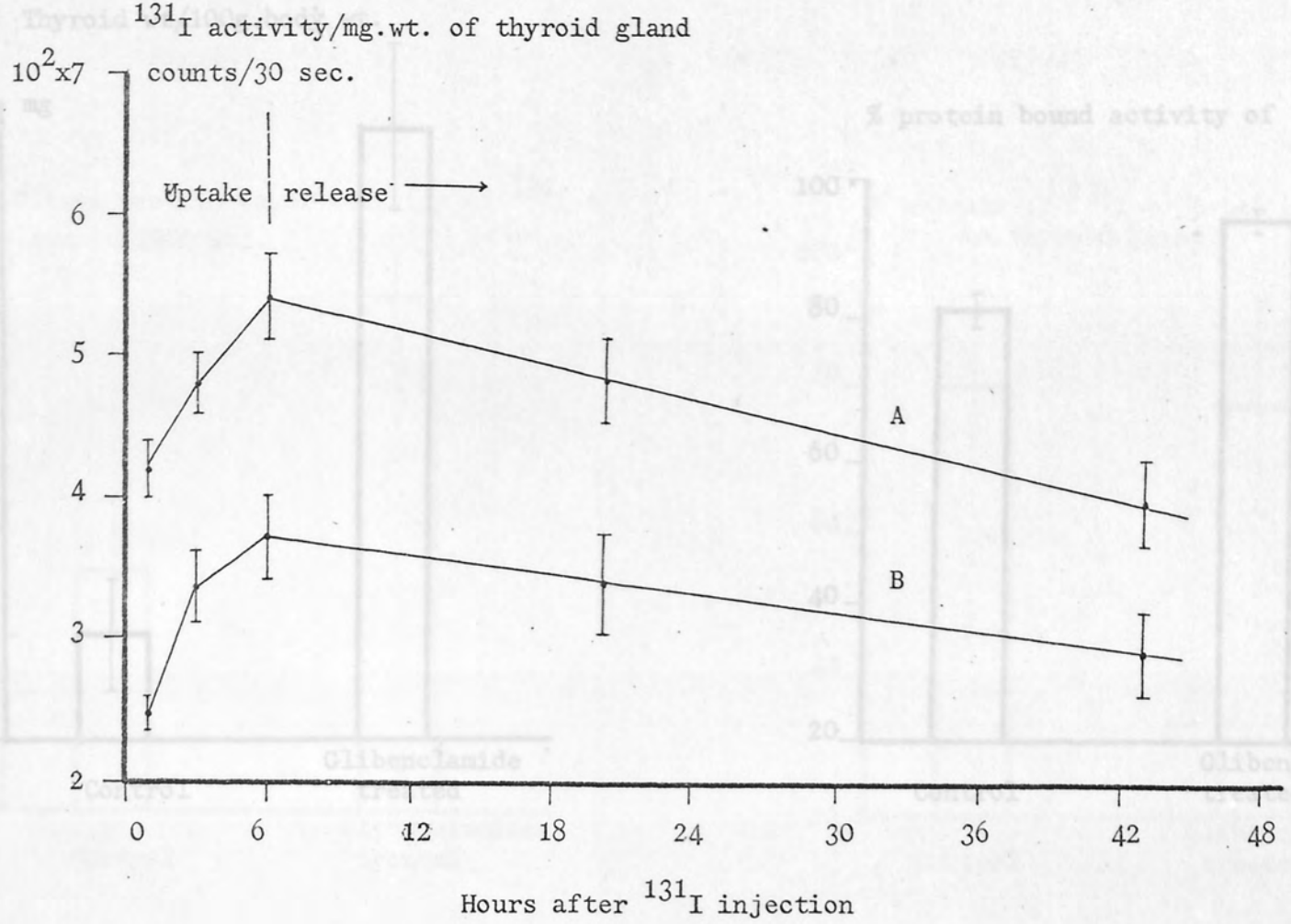

^{131}I activity/mg.wt. of thyroid gland
10^2x7 counts/30 sec.
Uptake
release
6
5
4
3
2
A
B
0
6
12
18
24
30
36
42
48
Hours after ^{131}I injection

Fig.42

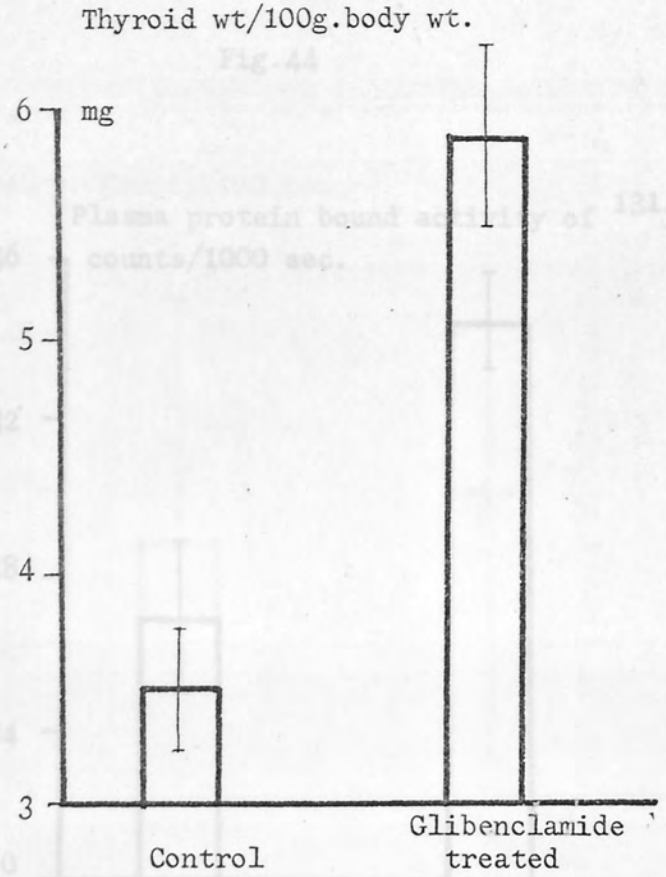

Thyroid wt/100g.body wt.
mg
6
5
4
3
Control
Glibenclamide treated

Fig.43

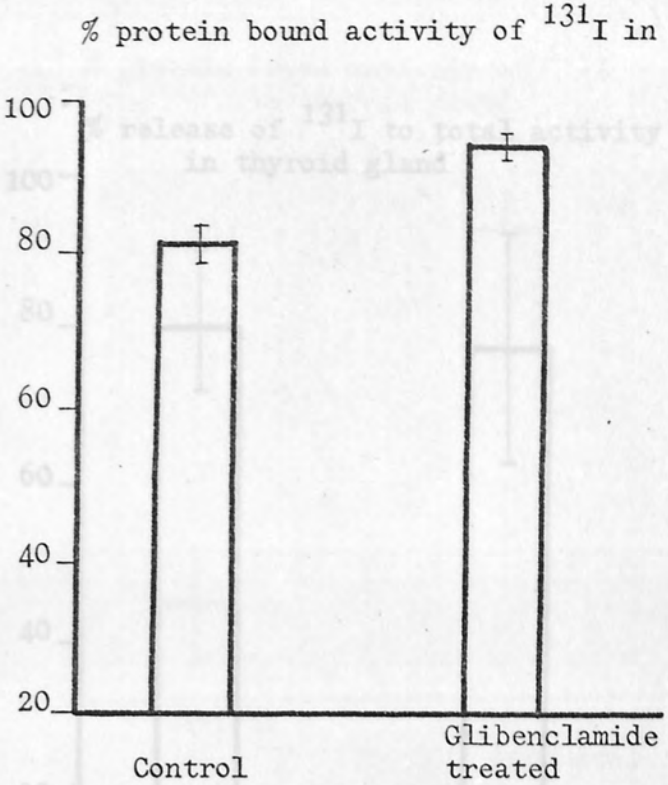

% protein bound activity of 131I in thyroid
100
80
60
40
20
Control
Glibenclamide treated

Fig.44

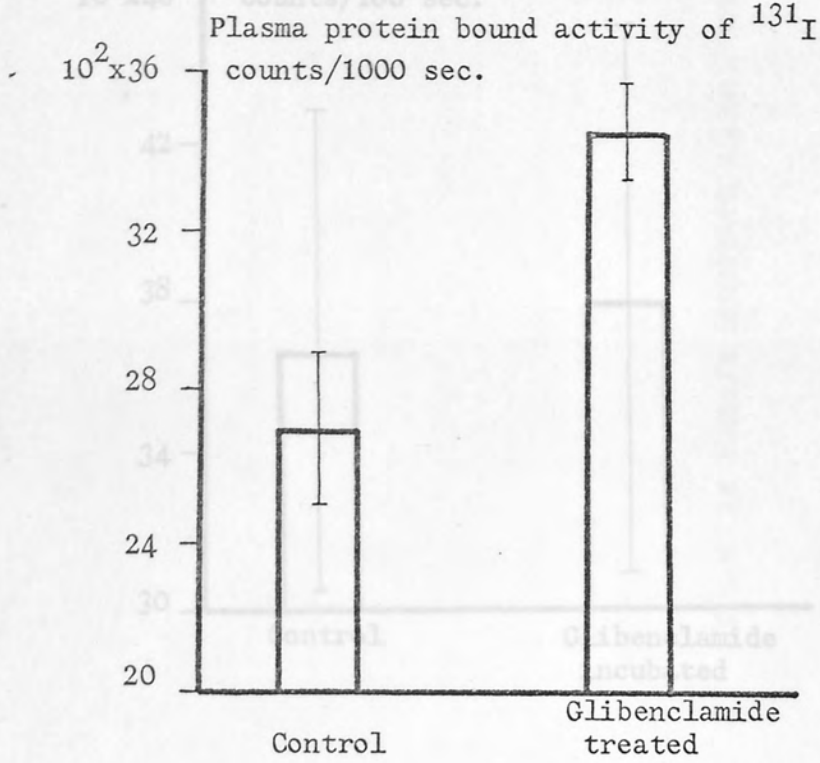

Plasma protein bound activity of ^{131}I
10^2x36
counts/1000 sec.
32
28
24
20
Control
Glibenclamide
treated

Fig.45

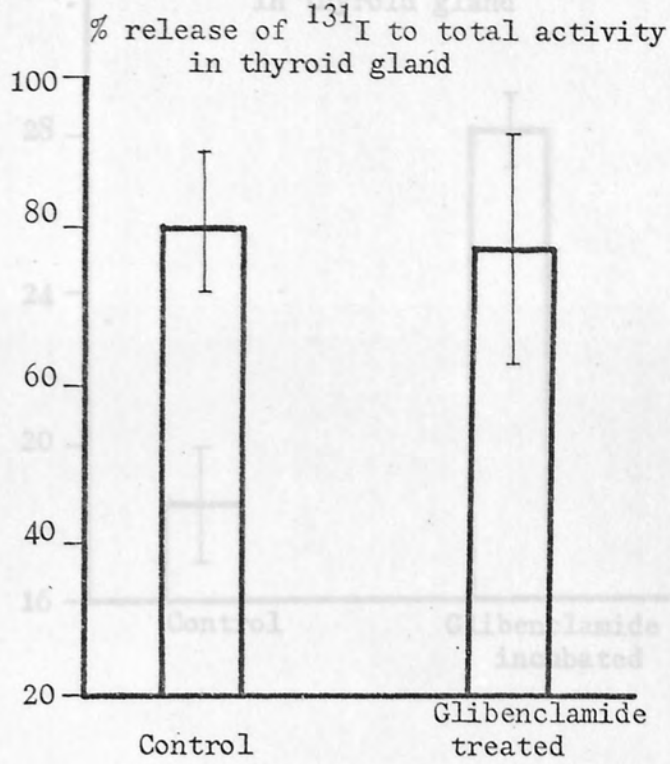

% release of ^{131}I to total activity
in thyroid gland
100
80
60
40
20
Control
Glibenclamide
treated

Fig.46

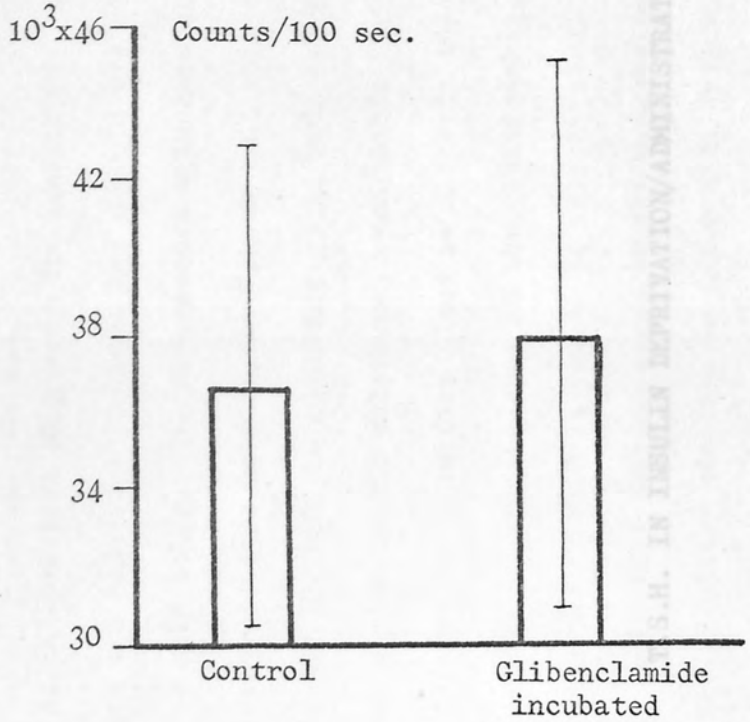
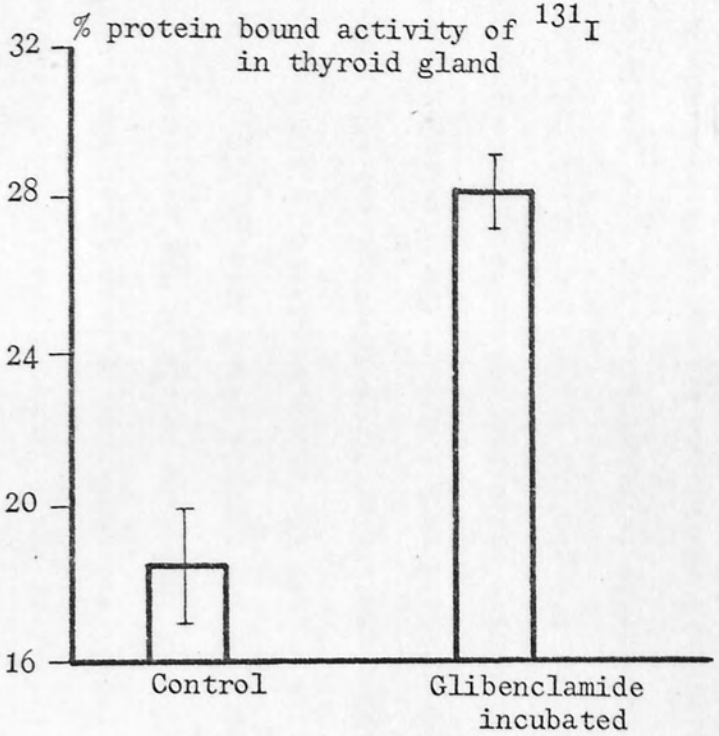

10^3x46
Counts/100 sec.
42
38
34
30
Control
Glibenclamide incubated

Fig. 47


% protein bound activity of ^{131}I in thyroid gland
32
28
24
20
16
Control
Glibenclamide incubated

T.S.H. IN INSULIN DEPRIVATION/ADMINISTRATION

INTRODUCTION

In the previous chapters, the experiments with insulin have shown that the endocrine pancreas and thyroid are not directly related to each other. The experiments with insulin deprivation like alloxan diabetes have further shown that this relationship is most probably through the pituitary gland mediated by some goitrogenic-like action of the substances responsible for hyperketonemia.

Whether the pituitary gland is directly involved or indirectly through hypothalamus in the relationship of the endocrine pancreas and the thyroid is yet to be investigated. However, there is little doubt that certain areas in the hypothalamus and anterior lobe of the pituitary comprise an integrated system regulating the activity of the thyroid gland. There are several data which indicate that a simple feedback system may control the pituitary-thyroid axis. Von Euler and Holmgren (260,261) using intrapituitary thyroxine microinjections, demonstrated the direct sensitivity of anterior pituitary cells to local changes in thyroxine concentration and this result was confirmed by Mess (262) who showed that the augmentation of thyroxine levels in the anterior pituitary decreases the activity of the thyroid gland left in situ. Implantation of thyroid tissue into the anterior pituitary had been shown to inhibit the formation of "thyroidectomy cells" in the hypophysis of thyroidectomized rats (263). The possibility that the anterior pituitary cells respond directly to thyroxine is also shown by the data of D'Anglo and Young (264) which indicate that thyroxine when systematically injected into rats with hypothalamic lesions, increases adrenal weight and plasma corticosterone levels. These

data indicate that thyroxine can simultaneously reduce the function of thyrotropic cells and enhance the secretory ability of the ACTH-producing elements.

The regulation of the TSH-thyroid system also presents examples which suggest the existence of a 'neuro-hormonal', 'negative' feedback mechanism. Lesions located in the anterior basal hypothalamus have been shown to inhibit the compensatory hypertrophy of the remaining thyroid lobe which usually follows hemithyroidectomy (265). Even more spectacular is the inhibition of the goitrogen effect of thiouracil treatment in animals bearing hypothalamic lesions (266, 267, 268). It is suggested that increase in the thyroxine concentration acts directly at the level of anterior pituitary and brings about a reduction of TSH secretion; on the other hand the decrease of the thyroid hormone levels induced either by hemithyroidectomy or by thiouracil administration stimulates TSH secretion by an effect which requires the participation of brain structures i.e. hypothalamus (269, 270).

Under the above-mentioned considerations the work presented in this chapter was performed to see if any effect on the thyrotropic state of the pituitary gland occurred while observing any changes in TSH levels in the blood under conditions of deprivation or excess administration of insulin in intact rats.

The history of the development of assay methods for TSH may be classified as gravimetric, histological and biochemical. The assay methods which are sensitive enough to detect the amount usually present in the blood and the pituitary of normal animals are either bioassay or radioimmunoassay methods. There are several bioassay methods for thyroid stimulating hormone (TSH).

For the measurement of TSH in pituitary extracts, several established procedures are quite satisfactory because a high degree of sensitivity is not required (271). However, for studies on the control of thyroid function it is necessary to be able to observe changes in the level of circulating TSH. In spite of many attempts, no widely accepted method for the assay of TSH which is sufficiently sensitive to detect the levels found in normal human serum has yet been described (272).

For many other hormones it is possible that radioimmuno-assay will provide satisfactory measurements of levels in human blood, e.g. insulin, parathyroid hormone, adrenocorticotrophic hormone and human growth hormone (273). This approach has been satisfactorily tried in laboratory animals and immunological similarity between bovine and rat TSH has enabled several investigators to develop radio-immuno-assays capable of measuring TSH in rat plasma (274,275,276). However, for human TSH this approach is unlikely to be satisfactory because knowledge of the chemistry of human TSH is scanty (277).

Various <u>in vitro</u> assays for TSH have already been reported, but the parameter for measuring the effect of TSH has not always been related to the known physiological action of the hormone. Some workers followed the increase in weight of bovine thyroid slices incubated with TSH (278), others described an assay in which ^{131}I release from guinea-pig thyroid slices was stimulated by TSH (279) and Kirkham (280) described another type of assay using guinea-pig thyroid slices from goitrous animals which were treated with methylethiouracil for 88 - 100 days.

Brown and Munro (281) described an assay method of TSH in

in which mouse thyroid tissue labelled with ^{131}I *in vivo* is transferred to an organ culture medium and the influence of TSH is studied *in vitro*. The parameter of TSH action is the release of ^{131}I from the thyroid gland into the medium.

It is reported that difficulties have been encountered in attempting to measure TSH levels in human serum because of 'labile interfering factor' or non-specific material (281,282), which result in gross release of radioiodine, clearly not attributable to human TSH. The non-specific material affecting ^{131}I release is removed from human serum and other preparations by filtration through 'Sephadex G-25' medium (282).

In this work an attempt was made to employ the technique used by Brown & Munro (281) after specific modifications in the general method.

MATERIALS AND METHODS.

TSH Assay.

Animals: Female white Swiss mice from an inbred strain sensitive to TSH in the McKenzie assay (287) were used. They were fed on Remingtons low iodine diet for at least 8 weeks prior to being used for assay.

Culture fluid: This was a modification of Gey's solution, closely resembling the medium used by Bottari et al. (279). Two stock solutions called chloride and phosphate solution were made up and stored at $4^{\circ}C$ (280). The salts, of 'Analar' grade, were dissolved in deionized millipored water. The chloride solution consisted of the following components: NaCl, 4.0 gms; KCl, 1.9 gms; $CaCl_2$(anhydrous), 0.65 gms; $MgCl_2 6H_2O$, 1.05 gms; and dissolved in 500 ml. of water. The phosphate solution consisted of the following

components: KH_2PO_4, 0.125 gms; $NaHPO_4$, 0.60 gms and were dissolved in 500 ml. of water. These solutions were sterilized in 20 ml. portions in screw topped glass containers by autoclaving at 20 lb./ sq. in. pressure for 20 min. Before use 20.0 ml. each of chloride and phosphate solutions were mixed and diluted to 100 ml. with deionized millipored water and the following components: glucose, 500 mg.; sodium bicarbonate, 300 mg.; potassium perchlorate, 14 mg.; bovine serum albumin (fraction v, powder), 500 mg.; penicillin G, 5 mg.; Streptomycin, 5 mg.; phenol red, 5 mg.; were added to 100 ml. of medium.

Standard TSH preparations

The standard TSH from Armour Pharmaceutical Co. was dissolved and diluted in Gey's solution. Fifty ml. of the standard preparation of 50 mμ/ml.TSH in the Gey's solution was filtered through "Sephadex G-25 medium". Nearly 15 ml. of early elute was discarded and the next 25 ml. elute was utilised for the other standards by diluting in the required amount of the Gey's solution. Standards ranging from 0.025 mμ/ml. to 1.000 mμ.TSH were prepared for the dose response curve.

Sensitivity and the effect of low iodine diet

The significant difference of the release of radioiodine from the control thyroids and the thyroids in the minimum dose of TSH was studied in three experimental procedures. The mice were kept on low iodine diet for 2, 5 and 8 weeks in the different experiments.

Serum from alloxan diabetic rats

Thirty rats were treated with 150 mg/kg. of body wt. of alloxan. The urinary blood sugar level was estimated; after four

days eleven rats had developed severe diabetes. The rest of the rats were further treated 100 mg/kg of alloxan and eight more developed severe diabetes. The rest were further treated with 100 mg/kg. of body wt. of alloxan. In two weeks twenty-one rats had developed severe diabetes. The rest either died or could not develop diabetes so were abandoned. After a week the blood sugar levels of the eighteen surviving rats were estimated, the blood sugar levels ranged from 384 mg% to 540 mg%. These rats in the induced diabetic condition were kept for a further five weeks.

Thirteen rats survived to the end of the period. These were killed by a blow on their head and blood serum collected in separate sterile tubes.

Serum from insulin treated rats.

Ten rats were kept under the same conditions as the alloxan diabetic rats and on the same day that the alloxan rats were killed, these were injected with 1 I.U/kg. body wt. of insulin intravenously in the tail vein. Eight hours after the injections the animals were killed and the blood serum was collected in separate sterile tubes.

Serum from control rats.

Twelve rats were kept under the same conditions as the alloxan diabetics and killed the same day. The blood serum was collected in separate sterile tubes.

Serum gel filtration.

The sera from the control rats were pooled into four groups, each being a pool of three rats; the sera from alloxan-diabetic rats were pooled in five groups, three groups pooled from three rats and two from two rats and the sera of insulin treated were pooled in four groups, two from three rats and two from two rats.

The groups of the pooled sera were passed through the 'Sephadex G-25' medium separately. In the group pooled from three rats, from about 9.0 ml. of serum only 4 to 5 ml. of middle elute was collected, and in the group pooled from two rats, from about 6 ml. of pooled serum, 2 - 3 ml. of middle elute was collected.

Assay Method.

Disposable sterilised test tubes with air tight caps were used. In a group of six tubes the same standard of TSH in 0.5 ml. or 0.5 ml. of serum from any group was pipetted and 0.5 ml. of normal medium was added. The air of the test tube was replaced by 95% O_2 and 5% CO_2 and tubes were sealed with caps.

Mice were injected (I-P) with 0.2 µci of ^{131}I ($Na^{131}I$) in 0.2 ml. of isotonic saline. Four hours after the injection of ^{131}I a group of six mice were killed by a blow on the head. The thyroid glands were removed still attached to a segment of trachea and quickly transferred to the prepared tubes. Each of the tubes of the group of six were reflushed with 95% O_2 and 5% CO_2, placed in a water bath at 37°C and shaken at 120 cyc/min for the required period, which was four hours for most of the experiments.

At the end of the incubation period the thyroid gland preparation was lifted gently from the culture medium, with one arm of a forcep and put in a separate test tube. The tube containing the medium was inserted in a well of a scintillation counter for the measurement of radioactivity released into the medium. The activity in the thyroid gland was measured separately and added up with the activity released, for total radioactivity in the thyroid gland at the beginning of incubation.

The release of ^{131}I from the thyroid gland into the medium

was then calculated and expressed as a percentage of the total radioactivity in the thyroid gland at the beginning of incubation.

Each of the different doses of TSH in the dose response curve were run on at least ten thyroids. Each of the groups of serum from all the control, alloxan diabetic and insulin treated rats were run on three to six thyroids.

RESULTS.

Fig. 48 shows the percentage release of ^{131}I from the incubated thyroids of mice kept on low iodine diet for two weeks against the different doses of standard TSH giving a dose response curve. In general the dose response curve was not satisfactory, particularly the thyroids incubated in the lower concentrations. TSH failed to show any significant difference in the percentage release of ^{131}I at these low concentrations (0 - 0.05 mU).

Fig. 49 shows the percentage release of ^{131}I from the incubated thyroids of mice kept on low iodine diet for five weeks against the different doses of TSH. The dose of 0.05 mU TSH significantly increased the release of ^{131}I from the thyroid incubated in the control medium or without TSH, but the dose of 0.025 mμ TSH failed to show any significant difference. In general the dose response curve improved from the previous experiment shown in Fig.48.

Fig.50 shows the dose response curve of the different doses of the standard TSH based on the percentage release of ^{131}I from the thyroids of mice kept on low iodine diet for eight weeks. The dose response curve significantly improved and seemed to be quite satisfactory. The doses of 0.025 and 0.05 mμ TSH significantly increased the release of ^{131}I from the thyroids of these mice from the thyroids incubated in the control medium or without T.S.H.

Fig.51 shows the percentage release of ^{131}I from each thyroid gland incubated in the sera of control, alloxan diabetic and insulin treated rats on the dose response curve. The TSH level ranged from 1.3 to 10.7 mμ; of alloxan diabetics from 6.6 to 46.0 mμ and of insulin treated rats from 15 mμ to 110 mμ/100 ml. of the serum.

Fig.52 shows the averages of the TSH levels in the sera of the control, alloxan diabetic and insulin treated rats. The TSH levels in the alloxan diabetic and insulin treated rats significantly increased from the control rats (alloxan diabetic $P < .05$, insulin treated $P < .05$).

DISCUSSION

The elevation of TSH levels in both exogenous insulin treated and alloxan diabetic rats proved a definitive involvement of the anterior lobe or thyrotrophic part of the pituitary gland in the alteration of the thyroid gland activity during these states.

In the alloxan diabetics hypoglycemia could be responsible for the elevated levels of TSH. Direct assay of plasma drawn from hypothyroid individuals has shown that frequently the concentration of TSH is higher than in clinically euthyroid individuals.Where it was not possible to demonstrate TSH in euthyroid plasma by direct assay, it was readily detectable in hypothyroidism and in several subjects where the TSH level ranged about 2.5 mμ/ml., the value fell from 2.25 mμ/ml. to 0.25 mμ/ml. after thyroxine therapy (283). Similarly other workers have found elevated values between 2 - 8 mμ in untreated hypothyroidism and thyroxine treatment restored the level to the normal value of about 0.2 mμ/ml.(284).

It is thought that the pituitary and thyroid gland interact through a simple feedback mechanism in which a lowered level of

thyroxine would stimulate the inhibited pituitary, which would secrete more TSH in an attempt to raise secretion of thyroxine by the thyroid.

It has already been shown and discussed previously that products characteristic of hyperketonemia through their goitrogen activity induce a hypothyroid condition. This hypothyroid condition brings an interaction in the feedback mechanism and elevates the TSH level.

In the exogenous insulin treated rats the stimulated state of the thyroid gland could be attributed to the elevation of the TSH level in the blood. The increased production of TSH after insulin administration either by its direct action on the pituitary or through the hypothalamus results in the increased release of thyroid hormones. It is well accepted that exogenous TSH increases the release of radioiodine from the thyroid gland in vivo (36). However, the feedback mechanism seems to interfere in the regular stimulation of the thyroid gland by insulin as, after the increased release of ^{131}I from the thyroid gland, sudden inhibition was observed in in vivo experiments with few doses.

Daily administration of insulin for a long period could have induced a sort of hyperthyroid state but it seems unlikely as no apparent hyperthyroid symptoms were observed and it shows that the thyroid gland activity is well controlled by feedback mechanism under the exogenous administration of insulin.

Fig.48. T.S.H.dose response curve of the percentage in vitro release of ^{131}I from the thyroid gland of the mouse kept on low iodine diet for two weeks.

Fig.49. T.S.H.dose response curve of the percentage in vitro release of ^{131}I from the thyroid gland of the mouse kept on low iodine diet for five weeks.

Fig.50. T.S.H. dose response curve of the percentage in vitro release of ^{131}I from the thyroid gland of the mouse kept on low iodine diet for eight weeks.

Fig.51. Percentage in vitro release of ^{131}I from each thyroid gland of the mouse incubated in the culture medium added with sera of alloxan diabetic, insulin treated and control rats.

Fig.52. The average of T.S.H.levels/100 ml. of serum of control, alloxan diabetic and insulin treated rats.

Fig.48.

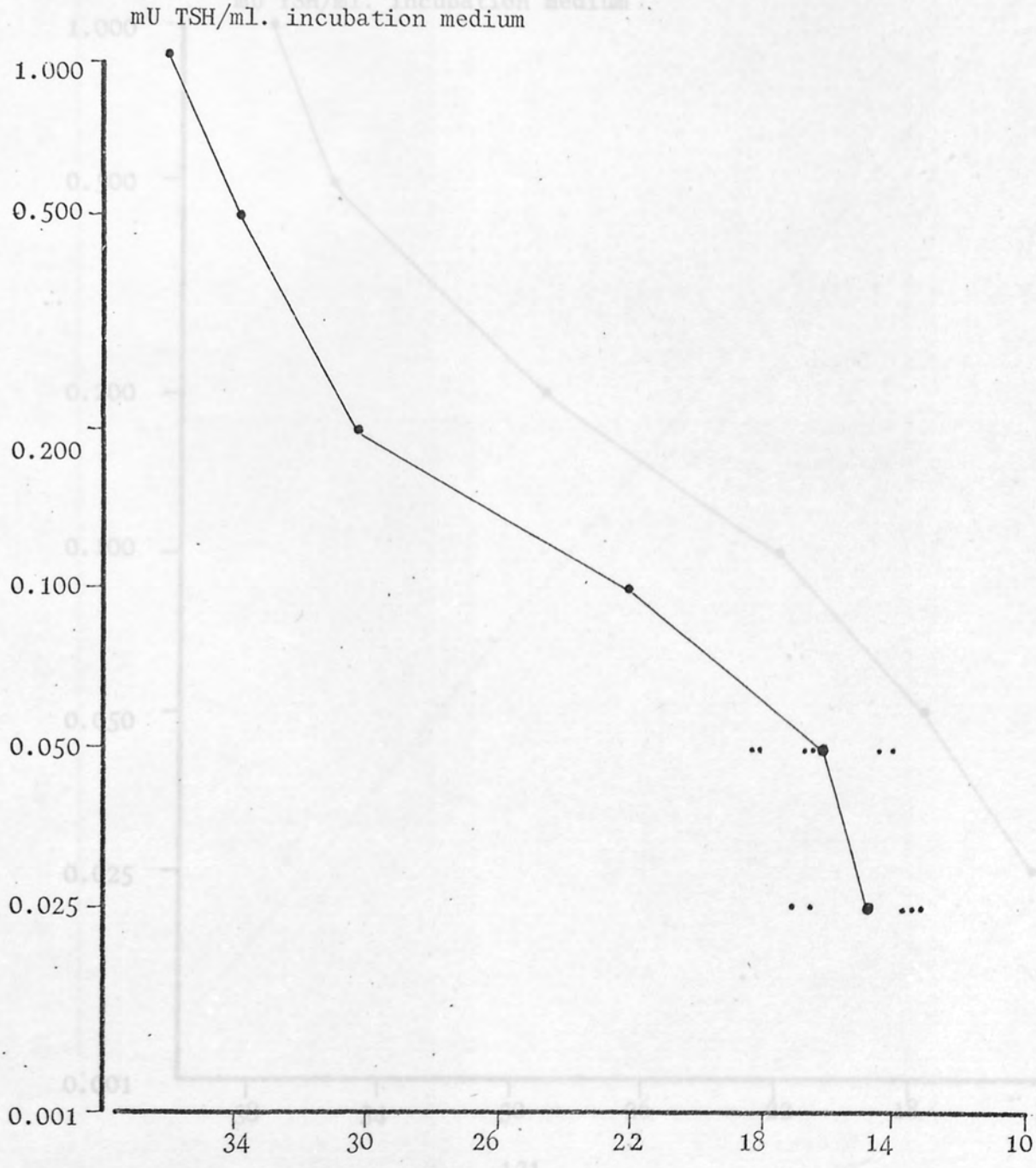
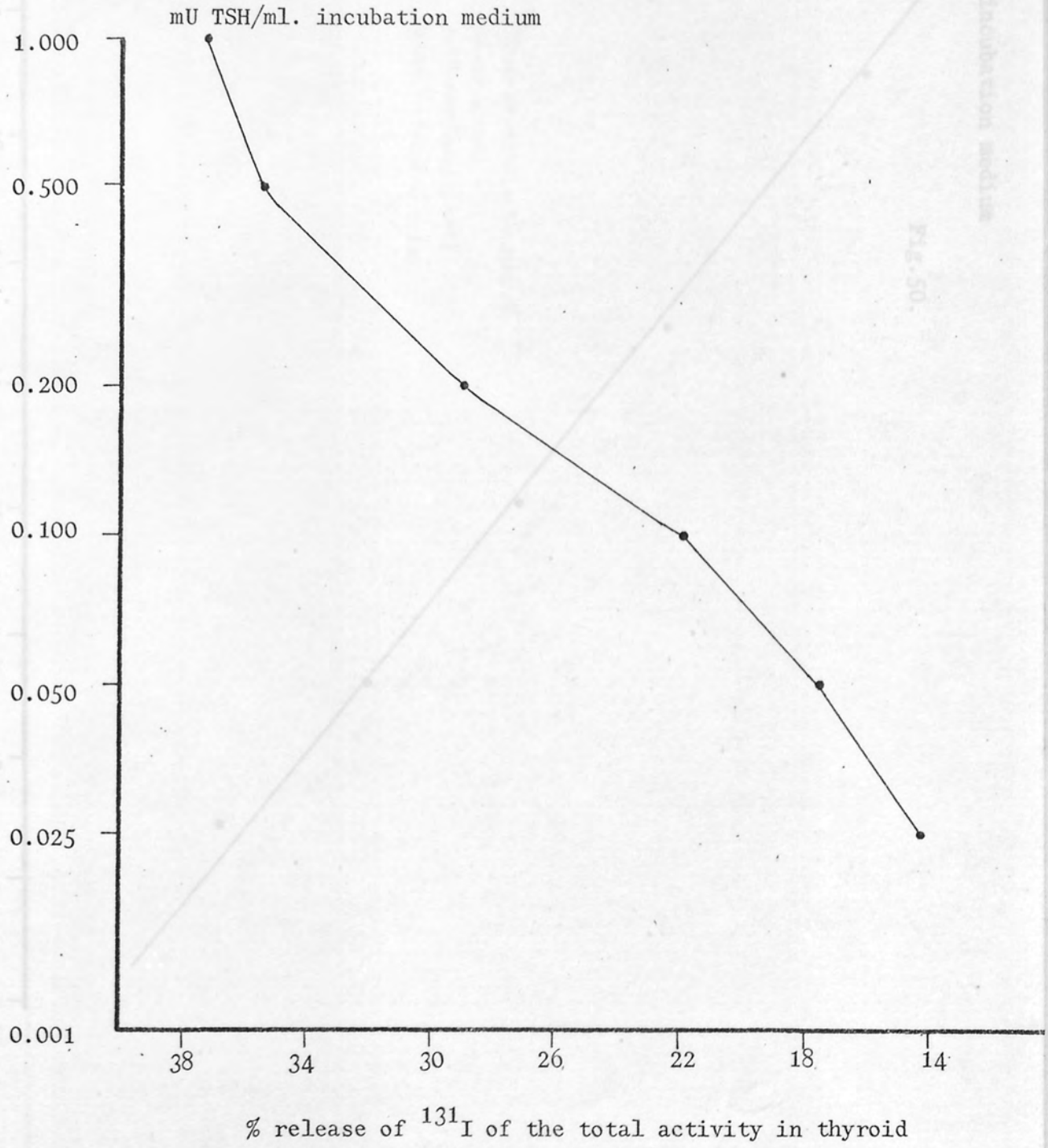

mU TSH/ml. incubation medium
1.000
0.500
0.200
0.100
0.050
0.025
0.001
34
30
26
22
18
14
10
% release of 131I of the total activity in thyroid

Fig.49.


mU TSH/ml. incubation medium
1.000
0.500
0.200
0.100
0.050
0.025
0.001
38
34
30
26
22
18
14
% release of ^{131}I of the total activity in thyroid

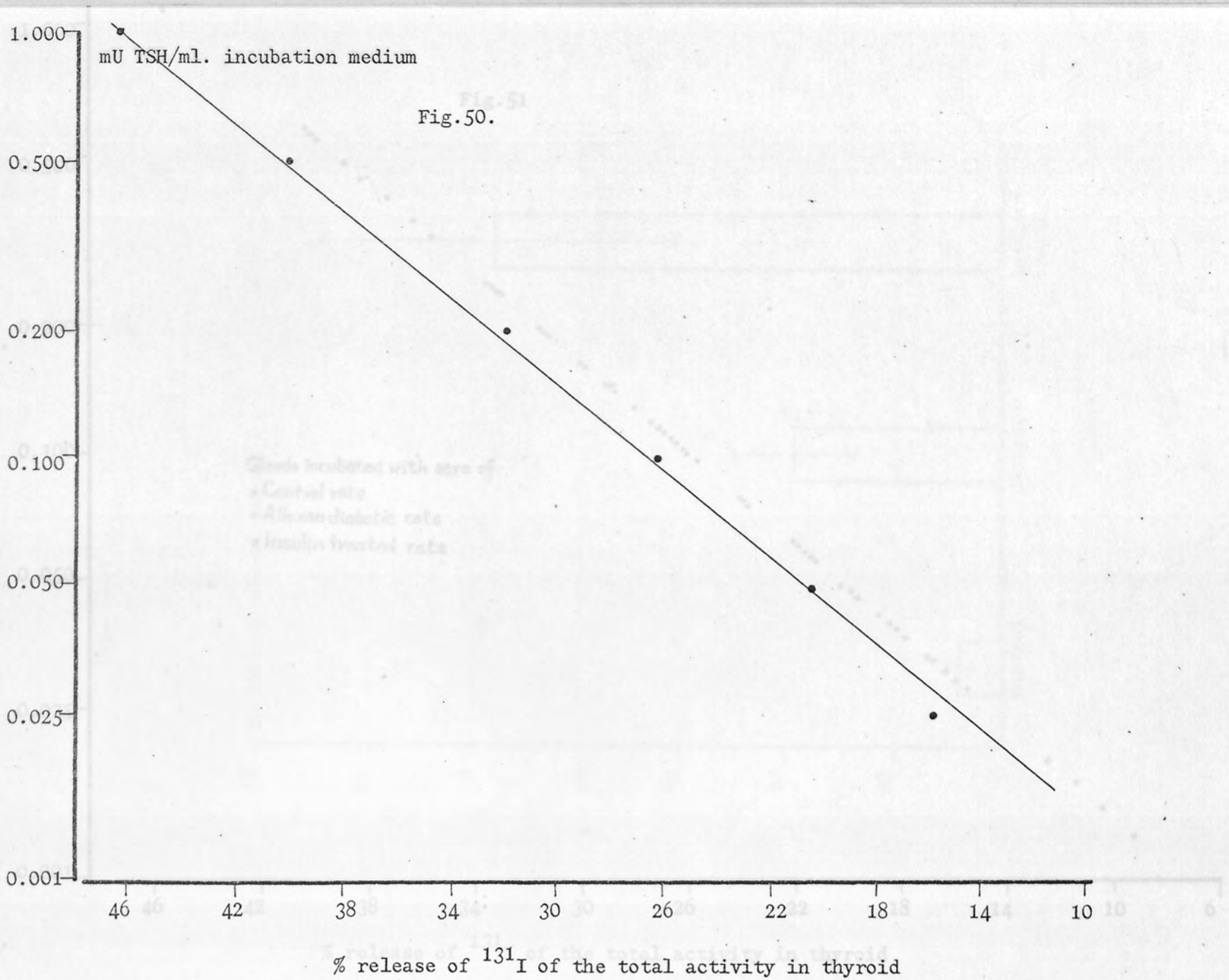

1.000
mU TSH/ml. incubation medium
0.500
0.200
0.100
0.050
0.025
0.001
46
42
38
34
30
26
22
18
14
10
% release of 131I of the total activity in thyroid

Fig.50.

Fig.51

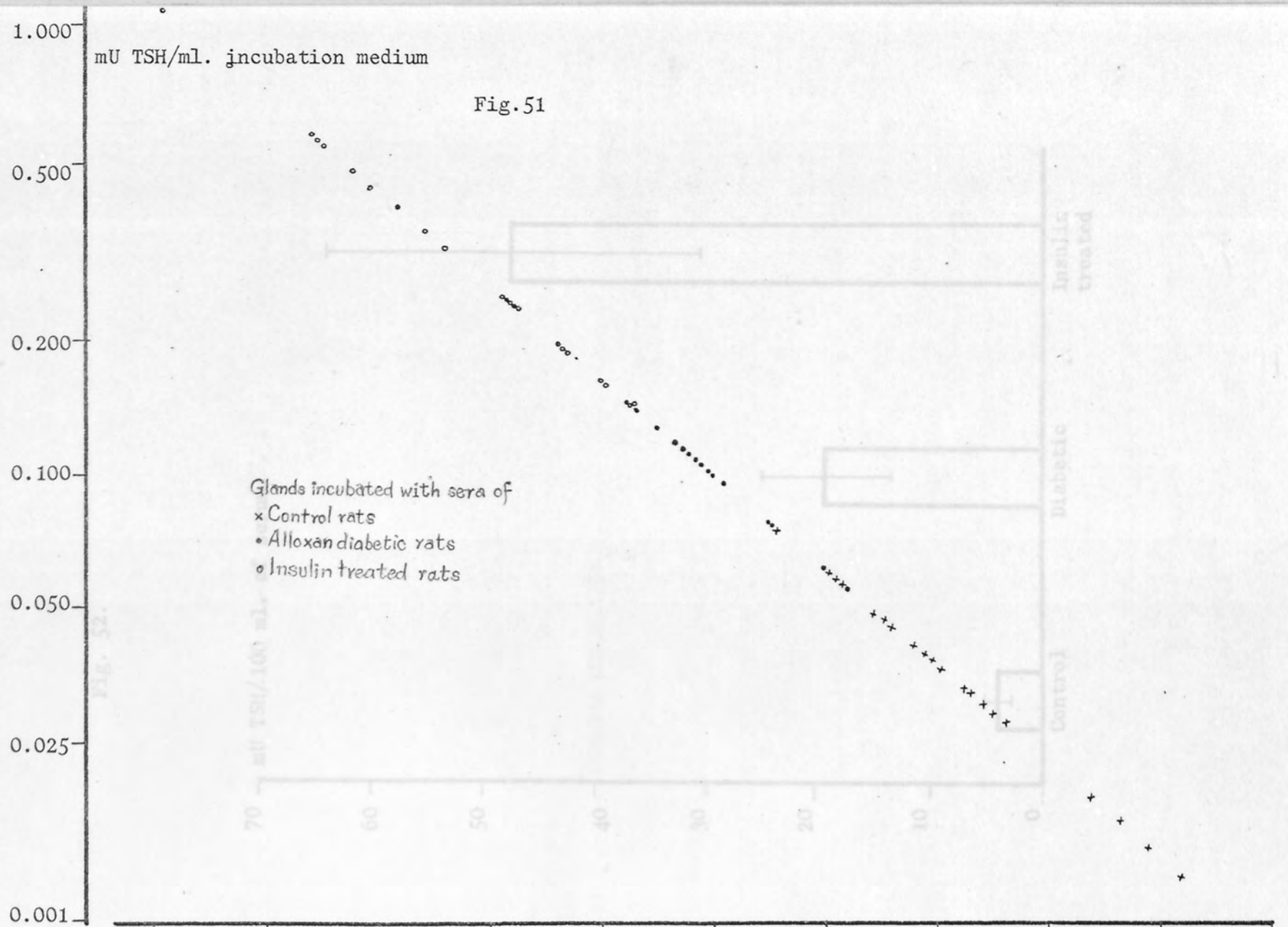
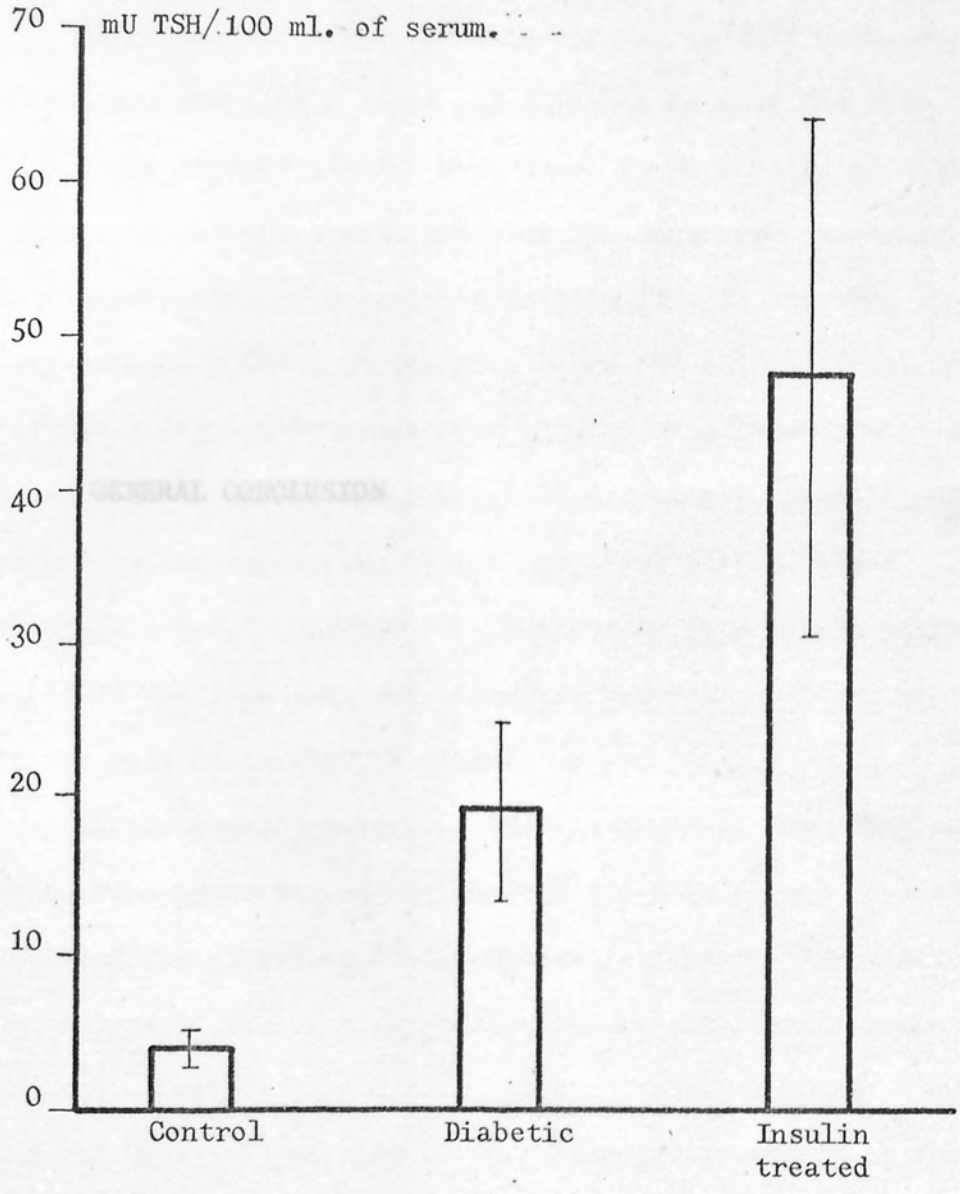

1.000
mU TSH/ml. incubation medium
0.500
0.200
0.100
0.050
0.025
0.001
Glands incubated with sera of
× Control rats
• Alloxan diabetic rats
○ Insulin treated rats
46
42
38
34
30
26
22
18
14
10
6
% release of 131I of the total activity in thyroid

Fig. 52.


70
mU TSH/100 ml. of serum.
60
50
40
30
20
10
0
Control
Diabetic
Insulin
treated

GENERAL CONCLUSION

The original stimulus for the work reported here stemmed from the observations that subnormal thyroid glands had been observed in the alloxan diabetic and insulin treated rats and some sort of pituitary role might be involved to influence and interfere with thyroid gland activity under insulin absence and excess administration.

It is apparent from the data present in this work that in the rat at least there is a close relationship between the endocrine pancreas and the thyroid gland. When there is an induced or pathological change in the functional state of the endocrine pancreas, a change in the thyroid gland activity follows it. It has been shown in the experiments in this thesis that where the altered functional states of the endocrine pancreas were induced by alloxan diabetes, partial and total pancreatectomy surgical procedures, insulin and hypoglycemic sulfonylureas administration, then thyroid gland activity was altered. However, the various in vivo and in vitro studies led to the idea that the endocrine pancreas activity was not directly related to thyroid gland.

In the considerations of the constancy of the relationship of the endocrine pancreas and the thyroid gland in normal conditions, the normal insulin secretion is the necessary factor. Any alteration in the normal secretion or its presence in the pancreas is responsible for the onset of crisis in the constancy. Conventionally, insulin maintains the constancy of many of the metabolic processes in the body and particularly in adipose tissue, muscle and liver. Drastic metabolic alterations are exhibited under the conditions of insulin deprivation or excess administration (285).

The main crises in the deprivation are the decreased level of insulin and production of the substances characteristic of hyper-

ketonemia, i.e. ketone bodies. The ketone bodies or at least one substance of these ketone bodies, acetoacetate, has proved in this work to be anti-thyroid in its characteristics. This antithyroid character is similar to the goitrogens, which interfere with the uptake of iodine and protein binding of iodine in the thyroid gland, as it has been shown in this work that acetoacetate decreases radio-iodine uptake by the thyroid and lowers protein binding of radio-iodine in the thyroid gland of the rat and mouse in vivo and in vitro. The decreased production of iodine hormone in the thyroid probably leads to inhibition of the release of the hormone from the thyroid gland. Furthermore it has been shown in this work that acetoacetate administration in vivo decreases the plasma protein bound ^{131}I of the rat.

The decrease in the insulin or deprivation of the insulin in alloxan diabetes could be another factor in the abnormalities of the thyroid gland activity. It has been suggested by Serif and Sihotang (36) that the depressed thyroid capacity of the alloxan diabetic rats may be due to a deficiency of endogenous TSH from the pituitary. This was in agreement with histological findings of de-granulation of acidophilic cells and decreased basophilic cell counts in the pituitaries of alloxan diabetic rats (45). Serif and Sihotang further suggested that decreased pituitary activity could be the outcome of alloxan injection since stress has been stated to induce a deficit of TSH and alloxan has been named as a stressor substance (45, 286); they themselves thought this to be unlikely as the diabetic rats injected with insulin had been subjected to the same stress of alloxan injection but TSH deficiency seemed to rehabilitate them. This would suggest that it is more the result

of the diabetic state than the direct action of alloxan.

Alterations in insulin, i.e. by partial deprivation or excess administration have shown an effect on the thyroid gland activity. The probable decreased levels of insulin in the partially pancreatectomised animals (in the absence of hyperketonemia) possibly caused the decreased thyroid hormone release rate, while increased levels of insulin induced by insulin administration led to the stimulation of thyroid hormone secretory rate. The experiments with insulin have shown that it does not affect the release rate of thyroid hormone in vitro, but it does in vivo. It can be suggested then that insulin does not affect directly the thyroid gland. Exogenous administration of insulin increased TSH levels in the blood and stimulated the thyroid to increase its secretory rate.

Thus it has been shown that the pituitary gland is definitely involved in the insulin and thyroid gland relationship. Other workers have shown that thyrotrophic cells of the anterior pituitary are under the influence of a thyroid stimulating hormone release factor (TSH-RF), this TSH-RF is released at the hypothalamus level (270). Are the thyrotrophic cells of the anterior pituitary which are directly or indirectly affected through the hypothalamus TSH-RF affected by alterations of insulin directly, irrespective of TSH-RF, or indirectly through the TSH-RF? This is the question yet to be investigated. There could be a possibility of direct effect as it has been shown that insulin enhanced the conversion of glucose-C^{14} to $C^{14}O_2$ and lipid in the slices of the rat anterior pituitary gland (173).

Similarly the involvement of the pituitary gland is quite likely in the alloxan diabetic, as shown in this work and perhaps

via the action of goitrogen-like ketone-bodies. TSH levels increased significantly in the alloxan diabetic. There are several data which indicate that simple feedback mechanism may control the pituitary thyroid axis. It has been demonstrated that there is a direct sensitivity of anterior pituitary cells to the local changes in the thyroxine concentrations (260,261,262). Decreased thyroxine level in the diabetic condition due to goitrogenic action of ketone bodies might be affecting the feedback mechanism and leading to an increase in TSH level. The action of the goitrogen activity of ketone bodies leading to an increase in the TSH level is further supported by the results in this work, as the weights of the thyroid gland increased in prolonged and severe diabetes in the rat, an action very similar to the goitrogen substances like thiourea and thiouracil etc. (257, 258, 259). However, the involvement of the hypothalamus cannot be ruled out here; the TSH-RF is regulated by neurogenic factors and by the changes of thyroxine concentrations at the hypothalamus (270).

Another factor which is worth consideration is that it is either insulin or the hypoglycemia induced under the effect of insulin which interprets the hypothalamus-pituitary-thyroid interaction in the endocrine pancreas and thyroid relationship. Blum et al. (43) have suggested that hypoglycemia is the factor responsible for such an interpretation.

Hypoglycemia sulfonylureas were considered to be useful for such interpretation but their dominent role as goitrogens failed to make such an approach practicable. However, <u>in vitro</u> studies with glucose do give an idea that blood sugar levels are not directly concerned with thyroid gland activity.

This work strongly suggests that the β-cells of the endocrine pancreas, the hypothalamus, the anterior lobe of the pituitary and the thyroid gland comprise an integrated system.

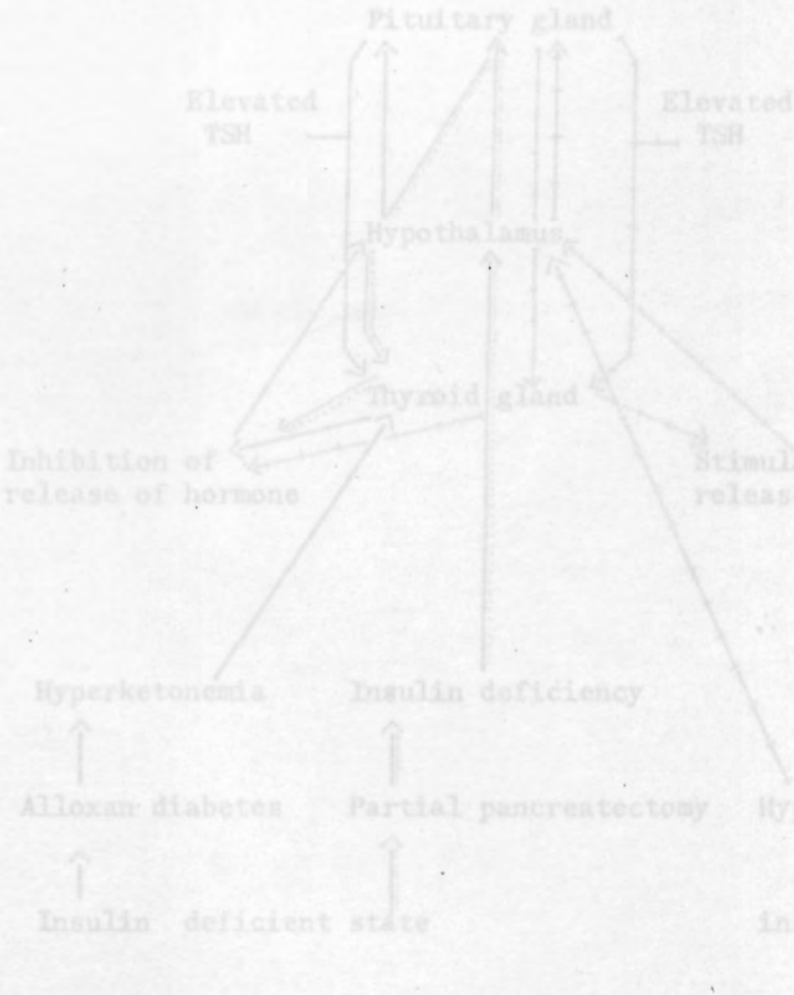
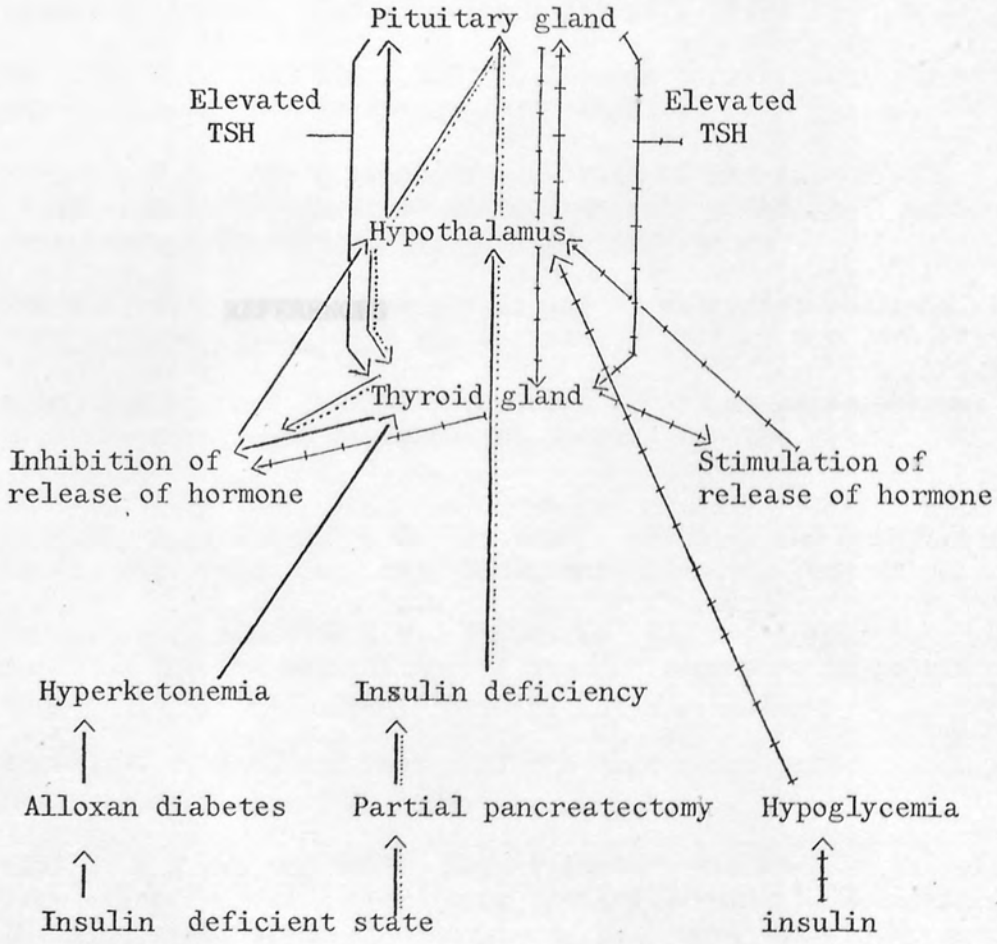


FIGURE A

Diagrammatic representation of the relationship of endocrine pancreas and thyroid gland

FIGURE A



Diagrammatic representation of the relationship of endocrine pancreas and thyroid gland

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