J. Endocr. (1974), **62**, 439–449
Printed in Great Britain

SHORT-TERM EFFECTS OF OESTRADIOL BENZOATE IN NORMAL, HYPOPHYSECTOMIZED AND ALLOXAN-DIABETIC MALE RATS

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(*Received* 15 *October* 1973)

SUMMARY

Studies were undertaken to determine the effects and possible mode of action of 17β-oestradiol benzoate (OEB) in alloxan-diabetic male rats. Ad-libitum or pair-fed normal, diabetic, and hypophysectomized rats received daily subcutaneous injections of 10 μg OEB for 10 days. In normal rats, OEB decreased plasma glucose, increased plasma immunoreactive insulin, growth hormone and corticosteroid levels, increased pancreatic β-cell granulation, and enhanced glucose stimulation of insulin release *in vitro*. In alloxan-diabetic rats, OEB treatment decreased urinary glucose excretion, increased plasma growth hormone and corticosteroid levels and slightly enlarged the pancreatic islets of Langerhans. In hypophysectomized rats, OEB decreased plasma glucose, increased plasma insulin levels, and slightly enlarged the pancreatic islets of Langerhans.

These results suggest that OEB affects experimental diabetes by a direct action on the pancreas, promoting insulin formation, and possibly by an indirect action mediated through hypophysial secretions.

INTRODUCTION

The beneficial influence of oestrogenic substances upon the incidence and nature of experimental diabetes in animals has been known for many years. Early studies in dogs (Barnes, Regan & Nelson, 1933) and monkeys (Nelson & Overholser, 1936) indicated that oestrogen administration reduced hyperglycaemia and glycosuria after 95% of the pancreas had been removed. Also, it has been demonstrated that the administration of natural and/or synthetic oestrogenic compounds to subtotally pancreatectomized or alloxan-diabetic rats ameliorates the diabetic condition and, if injected into diabetic rats over a long period of time, leads to a permanent 'protective' action including establishment of normal blood glucose levels (Rodriquez, 1954, 1965). Thus, it has been substantiated that oestrogenic compounds ameliorate the course of experimental diabetes. The mechanism by which oestrogen acts as an antidiabetic agent has still to be explained. To obtain information as to how oestrogen may alter the course of experimental diabetes in rats, the response of normal, hypophysectomized and alloxan-diabetic male rats to short-term oestrogen administration was studied.

MATERIALS AND METHODS

Animals

Adult male rats (Holtzman strain), 70–85 days old before experimental treatment, were housed singly (24 ± 2 °C; 12 h light) and provided with Purina laboratory chow and water *ad libitum* (except in paired feeding studies). Rats used for the diabetic study were fasted for 48 h before i.v. (tail vein) injection of recrystallized alloxan (33 mg/kg body wt), received no insulin replacement and were not used until 4 weeks after alloxan injection. Adult hypophysectomized rats were used 4 days after the operation and were fed a standard Purina laboratory chow supplemented daily with orange slices. The sella turcica was inspected at autopsy for completeness of hypophysectomy.

Hormone preparation and administration

Oestradiol benzoate (Nutritional Biochemical Corporation) dissolved in olive oil was injected s.c. once daily for 10 days. Doses of 10, 25 or 50 μg oestradiol benzoate in 0·1 ml olive oil were administered; control rats received the same volume of oil vehicle only.

Organ weights, tissue and plasma analyses

The rats were not fasted before being killed by decapitation, neck blood was collected in heparinized polyethylene centrifuge tubes, the blood was centrifuged and plasma was separated and stored at −20 °C until analysed. Blood and/or plasma glucose was determined by Somogyi's (1952) method, plasma protein by the method of Lowry, Rosebrough, Farr & Randall (1951), plasma corticosteroid by a modification of the method of Guillemin, Clayton, Lipscomb & Smith (1959), plasma immunoreactive growth hormone (GH) by the method of Birge, Peake, Mariz & Daughaday (1967) employing a rat GH as standard, and plasma immunoreactive insulin (IRI) by the double-antibody method of Hales & Randle (method C, 1963) utilizing an immunoassay kit (Amersham/Searle Company, Des Plaines, Illinois). Plasma IRI was expressed as μu./ml plasma, using human insulin as standard.

The anterior pituitary gland, left adrenal gland (trimmed), and seminal vesicle (fluid expressed) were removed, blotted, and weighed on a Roller-Smith torsion balance to the nearest 0·2 mg. A slice of liver was removed and frozen immediately between blocks of dry ice and stored at −20 °C until analysed for liver glycogen by the anthrone method of Seifter, Dayton, Novic & Muntwyler (1950). Urinary glucose collected daily from alloxan-diabetic rats was measured by the method of Somogyi (1952) and expressed as g glucose excreted/24 h.

Nucleic acids were extracted from individual anterior pituitary glands and pancreatic tissue by the method of Schneider (1957). RNA was determined by the phloroglucinol procedure (Miller, Golder & Miller, 1951) using Pabst yeast RNA as standard; DNA was determined by the diphenylamine method of Burton (1956) using sperm whale DNA as standard; total protein was determined by the method of Lowry *et al.* (1951).

Anterior pituitary gland water content was determined by drying the tissue in an oven at 105 °C for 24 h to obtain consistent successive dry weights.

Incubation of islets for determination of insulin secretion

Pancreatic islets were isolated by the technique of Lacy & Kostianovsky (1967). The medium used for incubation of the islets was a Krebs–Ringer bicarbonate solution containing 2 mg bovine plasma albumin/ml (Sigma), and either 30 or 300 mg glucose/100 ml. The medium (0·5 ml) was placed in incubation vessels and five islets were transferred to each vessel with an Irwin wire loop. The flasks were incubated in a Dubnoff metabolic shaker, 72 cycles/min at 37 °C for 90 min under a moistened atmosphere of 95 % O_2: 5 % CO_2 (v/v). Insulin assays were performed in duplicate on 0·1 ml aliquots of the medium after incubation, and results are expressed as μu. insulin released/five islets.

Electrophoresis and microscopy

Disc gel (polyacrylamide) electrophoresis was performed on anterior pituitary glands from control and diabetic rats by standard procedure (Davis, 1964). When light microscopy was employed, pancreatic tissue near the bile duct entrance was fixed, dehydrated, embedded in paraffin wax, sectioned at 4 μm, and stained with haematoxylin and eosin and aldehyde fuchsin. Electron microscopy was employed for the investigation of the splenic pancreas, the tissue being fixed, dehydrated, embedded in Maraglas, sectioned at 60–90 nm and examined with a Picker AEI microscope.

Statistical analyses

Statistical analyses were made using Student's *t*-test.

RESULTS

Effect of oestradiol benzoate on normal and alloxan-diabetic rats

Oestradiol benzoate at doses of 10, 25 or 50 μg/day for 10 days (Table 1) depressed the rate of increase in body weight, increased anterior pituitary and left adrenal gland weights, and decreased seminal vesicle weights in both normal and alloxan-diabetic rats. In addition (data not shown), OEB treatment decreased blood glucose levels and increased total anterior pituitary protein content, RNA content and concentration, DNA content, and the ratio of RNA:DNA in normal rats; blood glucose levels of alloxan-diabetic rats were not reduced significantly, while anterior pituitary protein content, RNA levels, and RNA:DNA ratios increased in this group. The changes in pituitary composition and content were not a result of alterations in water content.

The change in urinary glucose excretion by alloxan-diabetic rats injected with OEB was marked, while control diabetic animals excreted approximately the same amount of glucose each day during the 10-day injection period, irrespective of the initial levels of glycosuria. Levels of glucose excretion before and after the 10-day period in diabetic rats were ($\pm$ S.E.M.): controls 8·4 $\pm$ 0·7 *v.* 8·3 $\pm$ 0·6; 10 μg OEB-treated group 9·5 $\pm$ 0·4 *v.* 5·8 $\pm$ 0·6; and 50 μg OEB-treated group 8·9 $\pm$ 0·7 *v.* 4·3 $\pm$ 0·9 g/24 h, respectively. (No values for urine excretion were obtained in the 25 μg OEB-treated diabetic group.) The decrement in glucose excretion was not due to a reduction in food intake produced by OEB because glucose excretion in control

alloxan-diabetic rats pair-fed with the experimental animals was unaltered throughout the study.

Because of the effectiveness of the lower dose of OEB, 10 μg OEB/day were injected in all further studies, even though this dose of OEB represents unphysiological levels in female rats.

Table 1. *Effect of different doses of oestradiol benzoate (OEB) daily for 10 days on body and organ weights of adult normal and alloxan-diabetic male rats (means* ± S.E.M.)

A. *Normal adult male rats*

Group	Body wt (g)		Anterior pituitary (mg/100 g body wt)	Left adrenal gland (mg/100 g body wt)	Seminal vesicle (mg/100 g body wt)
	Initial	Change			
Three groups of control rats† (18)	235 ± 4	63 ± 3	3·20 ± 0·03	7·62 ± 0·23	153 ± 4
10 μg OEB/day (12)	227 ± 3	13 ± 4**	6·13 ± 0·26**	11·86 ± 0·25**	56 ± 7**
25 μg OEB/day (12)	235 ± 2	1 ± 4**	5·60 ± 0·22**	11·80 ± 0·39**	57 ± 4**
50 μg OEB/day (11)	235 ± 2	3 ± 4**	6·11 ± 0·21**	11·96 ± 0·22**	60 ± 4**

B. *Alloxon-diabetic male rats*

Controls (diabetic rats injected with olive oil) (6)	260 ± 7	9 ± 3	3·18 ± 0·09	10·78 ± 0·29	157 ± 12
10 μg OEB/day (7)	220 ± 19	−18 ± 5**	4·83 ± 0·36**	16·98 ± 1·77**	80 ± 6**
25 μg OEB/day (6)	228 ± 11	−11 ± 3**	4·85 ± 0·21**	15·88 ± 0·86**	66 ± 6**
50 μg OEB/day (7)	250 ± 19	−26 ± 4**	4·82 ± 0·39**	15·61 ± 1·90*	92 ± 9**

* $P < 0·05$ and ** $P < 0·01$ compared with the mean of the appropriate control group.

† This control group comprised three sub-groups of rats injected with olive oil, peanut oil, or not injected; statistical analysis of variance indicated no significant differences between the results from the three groups.

Number of observations in parentheses.

Effect of oestradiol benzoate and pair-feeding on body and organ weights and plasma hormone levels of normal and alloxan-diabetic rats

Since OEB administration decreased food intake in normal and alloxan-diabetic rats by 28·3 % and 18·6 % per day, respectively, it was necessary to determine if the reduced food intake significantly altered any organ weight or plasma hormone level. Pair-feeding experiments were carried out by supplying oil-injected control rats (normal or diabetic) with the equivalent amount of food consumed daily by rats receiving 10 μg OEB/day (normal or diabetic). Based on the numerous comparisons which can be made between the groups presented in Table 2, these results indicate that OEB injections had an effect (either direct or indirect) on the weights of various organs in addition to those changes which may have been the result of a reduction in food intake alone.

The effects of both food restriction (*ad libitum v.* pair-fed) and OEB on plasma hormone levels are presented in Table 3. Pair-feeding normal rats (Group 2) resulted in a reduction of plasma glucose, GH and IRI when compared with normal rats fed *ad libitum* (Group 1). Normal rats treated with OEB (Group 3) had lower plasma glucose, increased plasma GH (*v.* Group 2), IRI (*v.* Group 2), and corticosteroid levels (*v.* Groups 1 and 2). Pair-fed diabetic rats (Group 5) had lower plasma glucose,

protein, and GH levels than diabetic rats fed *ad libitum* (Group 4), but higher IRI and corticosteroid levels. Diabetic rats treated with OEB (Group 6) had increased levels of corticosteroid (*v.* Groups 4 and 5) and plasma GH (*v.* Group 4); however, the increase in GH was not significant, probably due to the variability within Group 5.

Table 2. *Effect of oestradiol benzoate (OEB, 10 μg/day for 10 days) and feeding regimen on body and organ weights of ad-libitum and pair-fed normal or alloxan-diabetic male rats (means* ± S.E.M.)

Group	Feeding regimen‡	Body wt (g) Initial	Body wt (g) Change	Anterior pituitary (mg/100 g body wt)	Left adrenal gland (mg/100 g body wt)	Seminal vesicle (mg/100 g body wt)
Control, normal rats + oil (6)	*Ad libitum*	247 ± 1	59 ± 3	2·64 ± 0·14	6·30 ± 0·19	126 ± 8
Control, normal rats + oil (6)	Pair-fed	227 ± 6	28 ± 3††	2·29 ± 0·08	7·42 ± 0·28*	128 ± 7
Normal rats + OEB (6)	*Ad libitum*	235 ± 5	3 ± 6**	4·07 ± 0·16**	9·66 ± 0·16**	64 ± 6**
Control, diabetic rats + oil (6)	*Ad libitum*	261 ± 16	37 ± 3	2·05 ± 0·12	8·03 ± 0·44	118 ± 7
Control, diabetic rats + oil (4)	Pair-fed	290 ± 25	16 ± 3††	2·10 ± 0·06	7·98 ± 0·70	140 ± 8
Diabetic rats + OEB (10)	*Ad libitum*	255 ± 8	11 ± 5††	3·59 ± 0·17**	11·85 ± 0·50**	73 ± 4**

* $P < 0·02$ compared with the mean of the appropriate control group fed *ad libitum*; ** $P < 0·01$ compared with the appropriate control pair-fed group; †† $P < 0·01$ compared with appropriate control group fed *ad libitum*.

‡ Two to four days after the start of OEB injections in the experimental groups, animals in the pair-fed groups received a daily ration equivalent to the food consumed by the experimental animals.

Number of observations in parentheses.

Effects of oestradiol benzoate and feeding regimen on adult hypophysectomized rats

Hypophysectomy and pair-feeding techniques were employed to determine whether the effects of OEB on body and organ weights and plasma hormone levels of both normal and diabetic rats were mediated through the pituitary gland (Table 4). Body weights of pair-fed hypophysectomized rats were not reduced significantly, although the weights of the adrenal glands and seminal vesicles were reduced significantly. The body weight of hypophysectomized rats treated with OEB was reduced significantly from control levels though the decreases in adrenal gland and seminal vesicle weights were similar to those of the pair-fed control rats. These results indicate that the effects of OEB on body weight are independent of pituitary function, whereas enlargement of the adrenal glands and diminution of seminal vesicles, as seen in normal and diabetic rats (Table 1) in response to OEB, are mediated through the pituitary gland.

The results presented in Table 4 also demonstrate the effects of OEB on the plasma components of hypophysectomized rats. Pair-fed hypophysectomized rats had lower plasma glucose, protein and IRI levels compared with rats fed *ad libitum*. Hypophysectomized rats treated with OEB had lower plasma glucose and protein levels and increased plasma IRI than those of the oil-treated control group. No

significant change in plasma corticosteroid levels was noted. These results indicate that for OEB to augment plasma corticosteroid levels, the pituitary gland must be present. In addition, OEB may act directly on the pancreas to augment plasma IRI levels.

Table 3. *Effect of oestradiol benzoate (OEB, 10 μg/day for 10 days) and feeding regimen on plasma glucose, protein, growth hormone (GH), immunoreactive insulin (IRI), and corticosteroid (Cort.) levels in normal and alloxan-diabetic male rats (means* ± S.E.M.)

(Degree of glycosuria measured on the first and last day of treatment of groups 4, 5 and 6 were: 6·56 ± 0·52 and 6·50 ± 0·68; 6·43 ± 2·04 and 7·10 ± 1·84; 9·75 ± 0·67 and 6·50 ± 0·96 (S.E.M.) g/24 h, respectively.)

Group number and treatment	Feeding regimen‡	Plasma components: Glucose (mg/100 ml)	Protein (%)	GH (ng/ml)§	IRI (μu./ml)	Cort. (μg/100 ml)
1. Control, normal rats + oil (6)	*Ad libitum*	153 ± 3	7·77 ± 0·06	61 ± 12	74 ± 5	15·1 ± 0·5
2. Control, normal rats + oil (6)	Pair-fed	139 ± 4**	7·94 ± 0·14	22 ± 10**	24 ± 4**	12·1 ± 2·1
3. Normal rats + OEB (6)	*Ad libitum*	142 ± 2**	8·76 ± 0·15***	71 ± 14††	34 ± 2***†	21·5 ± 0·7***†††
4. Control, diabetic rats + oil (6)	*Ad libitum*	564 ± 28	7·72 ± 0·41	46 ± 20	16 ± 4	8·3 ± 1·7
5. Control, diabetic rats + oil (4)	Pair-fed	429 ± 86	6·96 ± 0·12	18 ± 14	35 ± 5**	13·8 ± 1·6*
6. Diabetic rats + OEB (10)	*Ad libitum*	533 ± 73	7·50 ± 0·21†	48 ± 8	20 ± 6	19·2 ± 1·4***†

* $P < 0·05$; ** $P < 0·02$; *** $P < 0·01$ compared with mean of appropriate control group fed *ad libitum*.

† $P < 0·05$; †† $P < 0·02$; ††† $P < 0·01$ compared with mean of appropriate control pair-fed group.

‡ Two to four days after the start of OEB injections in the experimental groups, animals in the pair-fed groups received a daily ration equivalent to the food consumed by the experimental animals.

§ The growth hormone analyses were done by Dr W. J. Schindler, Baylor College of Medicine, Houston, Texas, U.S.A.

Number of observations in parentheses.

Nucleic acid determinations made on pancreatic tissue of hypophysectomized rats were compared with those obtained from normal and diabetic rats and indicated that hypophysectomized animals had depressed pancreatic protein levels and increased DNA levels. Injection of OEB increased pancreatic DNA levels in hypophysectomized and diabetic rats; OEB also increased RNA levels in the diabetic group. Light and electron microscopy indicated larger islets (hypophysectomized and diabetic groups) and increased β-cell granulation (intact and diabetic groups). Because of these alterations in tissue morphology in response to OEB, it was considered desirable to study the effects of OEB on islet cell insulin synthesis and/or release.

Table 4. *Effect of oestradiol benzoate (OEB, 10 μg/day for 10 days) in adult hypophysectomized male rats (means ± S.E.M.)*

A. *Body and organ weights*

Group	Feeding regimen§	Body wt (g) Initial	Body wt (g) Change	Left adrenal gland (mg/100 g body wt)	Seminal vesicle (mg/100 g body wt)
Control, oil vehicle only (6)	*Ad libitum*	186 ± 11	−4 ± 4	5·42 ± 0·28	45 ± 8
Control, oil vehicle only (9)	Pair-fed	178 ± 4	−13 ± 2	3·80 ± 0·17**	28 ± 2
OEB (8)	*Ad libitum*	186 ± 6	−33 ± 3‡‡	4·35 ± 0·25*	32 ± 2

B. *Plasma components*

		Plasma components: Glucose (mg/100 ml)	Protein (%)	Insulin (μu./ml)	Corticosteroid (μg/100 ml)
Control, oil vehicle only (6)	*Ad libitum*	147 ± 3	7·42 ± 0·08	36 ± 1	3·1 ± 0·3
Control, oil vehicle only (9)	Pair-fed	115 ± 4**	7·06 ± 0·07**	10 ± 2**	3·8 ± 0·2
OEB (8)	*Ad libitum*	102 ± 4‡‡	6·38 ± 0·15‡‡	28 ± 5††	3·3 ± 0·1

* $P < 0·02$, **$P < 0·01$ compared with mean of appropriate control group fed *ad libitum*; †† $P < 0·01$ compared with mean of appropriate pair-fed group; ‡‡ $P < 0·01$ compared with means of both control groups.

§ Two to four days after the start of OEB injections in the experimental group, animals in the pair-fed group received a daily ration equivalent to the food consumed by the experimental animals.

Number of observations in parentheses. The results in A and B were obtained from the same rats.

Table 5. *Effect of oestradiol benzoate (OEB, 10 μg/day for 10 days) administered to rats* in vivo *on isolated pancreatic islet insulin release* in vitro *(means ± S.E.M.)*

Group number and treatment	Number of observations	Glucose added to incubation medium (mg/100 ml)	Insulin release* (μu./5 islets)
1. Oil vehicle	8	30	308 ± 73
2. Oil vehicle	8	300	683 ± 31
3. OEB	7	30	324 ± 35
4. OEB	9	300	787 ± 12

* $P < 0·01$ for groups 2 *v.* 1, 4 *v.* 3 and 4 *v.* 2.

Effects of oestradiol benzoate on insulin secretion in vitro

Insulin release from isolated pancreatic islets *in vitro* was significantly increased in OEB-injected as compared with control rats when the glucose concentration in the medium was increased from 30 to 300 mg/100 ml (Table 5). Also, islet cells from normal rats treated with OEB released more insulin at higher concentrations of glucose in the media than did the islets from oil-treated control rats, indicating an insulin release-promoting effect of OEB.

DISCUSSION

Previous studies on the influence of oestrogenic compounds on the production and/or prevention of diabetes in rats were carried out after the administration of pharmacological doses of steroids for periods varying from 1 to 6 months (Rodriquez, 1965). Not all diabetic rats, however, respond to oestrogen administration; only in those rats with fasting blood glucose levels of less than 250 mg/100 ml was the diabetes ameliorated, possibly indicating that functional endocrine pancreas must remain after alloxan treatment for the rat to benefit from subsequent steroid injections. Although OEB markedly decreased glycosuria in diabetic rats in the present study, the short-term administration of OEB did not result in an amelioration of the diabetes with respect to changes in plasma glucose and IRI levels. In addition, it was noted that the less severely diabetic rats invariably tended to show the greatest decrease in glycosuria as well as the lowest blood glucose values at the end of the steroid treatment; yet, plasma IRI was not increased. It is clear that for oestrogen to be beneficial, it must be administered over an extended period and only to borderline or minimally diabetic animals. Nevertheless, the response of the diabetic animals to OEB was in many instances identical with that observed in normal and hypophysectomized rats. It is possible, therefore, that the effects of OEB in the various groups of experimental animals studied here are early stages in a sequence of events, which if treatment were continued over a longer period, could beneficially alter the course of the experimental diabetes.

The factors most likely to be involved in the ameliorative process are: (*a*) influence of OEB on food intake with concomitant reduction of the rate of body growth; (*b*) inhibition of testosterone secretion; (*c*) modification of anterior pituitary gland function and (*d*) effect on the pancreatic islets.

It is well known that oestrogen injections reduce food intake (Meites, 1949) and may suppress rate of body growth (Zondek, 1936; Ingle, 1941; Meites, 1949; Rodriquez, 1965), as well as that fasting and dietary restriction can control and/or improve the diabetic state both in laboratory animals and in man. The reduction of glycosuria and of body weight observed in the pair-fed control rats indicated that OEB exerted an effect(s) quite distinct from that depressing food intake. Therefore, it does not appear that depression of appetite and growth inhibition contributes significantly to the anti-diabetic effects of OEB injections.

That suppression of testosterone secretion by the administration of OEB may alter the course of experimental diabetes in rats is a suggestion supported by evidence indicating that (i) castration of male rats reduces the incidence of diabetes expected after subtotal pancreatectomy (Rodriquez, 1965); (ii) testosterone injections into ovariectomized pancreatectomized rats exacerbate pre-existing diabetic states in rats (Rodriquez, 1965), and (iii) administration of testosterone to castrated rats impairs tolerance to glucose loads (Bailey & Matty, 1972). Although plasma luteinizing hormone levels were not measured in the present study, the suppressive action of OEB on this hypophysial secretion is well established (Schiavi, 1968) and was probably most clearly indicated in our study by the marked atrophy of the seminal vesicles of the normal and diabetic, but not of the hypophysectomized, rats (compare Tables 1 and 2 with Table 4). The precise role played by testosterone in modifying

experimental diabetes awaits clarification, especially since the results presented indicate that OEB administration to hypophysectomized rats enhanced plasma IRI levels (Table 4B).

Since it has been established that the pituitary gland influences carbohydrate metabolism and the diabetic syndrome, its role must be considered in any explanation by which OEB may influence diabetes in rats. Alterations in the secretion of GH, corticotrophin (ACTH), and prolactin, by the rat anterior pituitary gland have been brought about by oestrogen and could produce pronounced changes in carbohydrate metabolism. Evidence has been presented indicating that receptor sites for oestrogen exist in the anterior pituitary gland of rats (Korach & Muldoon, 1973; Leavitt, Kimmel & Friend, 1973). In the present investigation, OEB significantly increased plasma immunoassayable GH levels in pair-fed normal rats; the same trend was observed in pair-fed diabetic rats (Table 3). Studies already published indicate that oestrogenic compounds increase plasma GH levels in man (Frantz & Rabkin, 1965; Spellacy, Buhi & Bendel, 1969*a*, *b*) and laboratory animals (Ito, Martin & Furth, 1970; Lloyd, Meares, Jacobe & Thomas, 1971). It has also been demonstrated that OEB increased plasma prolactin levels in male and female rats (Voogt, Chen & Meites, 1970). Although direct measurement of plasma prolactin levels was not made in the present study, increased width and stain intensity of prolactin bands in disc gel electrophoretic profiles were observed, indicating an augmentation of pituitary synthesis (and possible release) of prolactin. In addition, the classic study of Long, Katzin & Fry (1940) emphasizes the importance of the adrenal cortex in carbohydrate metabolism. The role of oestrogen in corticosteroidogenesis has been the subject of intensive investigation, and many findings point to increased plasma ACTH and adrenal steroid production (Kitay, 1968; Nelson, 1968; Wennhold, Lauro & Nelson, 1970; Kitay, Coyne & Swygert, 1971). In the present study, OEB increased plasma corticosteroid levels in normal and diabetic rats. This effect was mediated by the pituitary gland since hypophysectomized rats did not respond with an augmentation of plasma corticosteroid.

In addition to the increases in the levels of hypophysial hormone observed after OEB administration, injection of ACTH or corticosteroids *in vivo*, as well as GH and/or prolactin secretion by implanted pituitary tumours, increase the insulin content of the rat pancreas as well as plasma IRI levels (Haist, 1965; Martin, Alerblom & Garay, 1968; Martin & Friesen, 1969). These effects may be brought about by a direct or an indirect action on the pancreatic β cell.

As already stated, the condition of severely diabetic rats (i.e. those with little or no functioning endocrine pancreas) showed no improvement in response to oestrogen administration. It has been reported that long-term OEB administration produces pancreatic islet hypertrophy and hyperplasia in normal, hypophysectomized or diabetic rats (Rodriquez, 1954, 1965) and increases pancreatic insulin content in normal rats (Haist, 1965). In addition, it has been reported recently that the long-term treatment of ovariectomized rats with OEB augmented plasma IRI levels (Basabe, Chieri, & Foglia 1969), and enhanced glucose-stimulated insulin secretion from isolated pancreatic islets of intact female rats (Costrini & Kalkhoff, 1971). It is noteworthy that these changes are common during late gestation in normal rats (Malaisse, Malaisse-Lagae, Picard & Flament-Durand, 1969; Costrini & Kalkhoff,

1971). The present investigation demonstrated enhanced plasma IRI levels in normal and hypophysectomized rats after short-term administration of OEB. Isolated pancreatic islets from normal rats treated with OEB *in vivo* displayed increased insulin release in response to glucose stimulation. Also, studies of pancreatic morphology showed cytological features indicating insulin release after OEB treatment. Thus, a β-cytotrophic action of OEB can be demonstrated even after a short period of administration (10 μg/day for 10 days).

It appears, therefore, that OEB administration for a short period augments β cell insulin release in rats. This action of OEB appears to be mediated directly through the endocrine pancreas, and indirectly through a modification of hypophysial release of GH, ACTH and prolactin. The response of the pancreas to the levels of the hormones in the peripheral circulation can possibly ameliorate the diabetic state if an adequate population of β cells is available, and if OEB is administered for a long enough period of time.

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