LXVIII. THE DIABETOGENIC ACTION OF CRUDE ANTERIOR PITUITARY EXTRACTS

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THE demonstration by Houssay & Biasotti [1931] that in a number of different species the symptoms of diabetes which normally follow pancreatectomy are greatly reduced by removal of the pituitary gland, has received ample confirmation. Houssay and his colleagues (whose work was summarized by Houssay [1936]) were further able to show that when crude anterior lobe extracts were administered to hypophysectomized-depancreatized animals, the symptoms of diabetes were greatly exacerbated, and that intact animals could be rendered diabetic by the injection of such extracts. This diabetogenic action of anterior lobe extracts in normal animals has also been demonstrated by other investigators [Evans *et al.* 1932; Baumann & Marine, 1932; Evans, 1933; Barnes & Regan, 1933; Nelson & Overholser, 1934], although negative results have been recorded in many instances [Shpiner & Soskin, 1934; Holden, 1934; Hrubetz, 1935; Long, 1936]. Houssay [1936] distinguishes two types of hyperglycaemic action exerted by anterior lobe extracts in intact animals; one, an immediate blood sugar-raising action [Eidelsberg, 1932; Lucke *et al.* 1933; Holden, 1934; Shpiner & Soskin, 1934; Elmer *et al.* 1937] and the other—the true diabetogenic action—exhibited only after repeated daily injections. The discovery that the administration of anterior lobe extracts can render normal animals diabetic has led to the suggestion that the pituitary gland elaborates a “diabetogenic hormone”, although there is no evidence to show whether this represents one, or more than one substance. Moreover, the use of the term “hormone” is most inapt, since the normal function of the factor concerned cannot be the production of a diabetic condition.

Because of the numerous negative findings, and because, in successful demonstrations, the true diabetogenic action of anterior lobe preparations is exhibited only after several daily administrations of large amounts of extract, the physiological significance of the diabetogenic action of these extracts in normal animals has recently been questioned [Chaikoff, 1936]. The investigations recorded in the present paper were undertaken in order to determine the conditions necessary for a consistent demonstration of the true diabetogenic action of pituitary extracts. It was hoped thus to throw light on the reason for the negative results recorded in the literature, and to provide a consistent physiological test which might be used in the concentration of the diabetogenic factor. During the course of these investigations it was found that in the dog the daily injection of a crude anterior lobe extract induced, in some cases, a diabetic condition that was apparently permanent. This observation was briefly reported in a preliminary communication [Young, 1937, 2].

The influence of anterior lobe extracts on the glycaemic response to injected insulin [Young, 1936; 1937, 1] is not dealt with in the present communication but is fully discussed in another paper. The present investigation is concerned

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only with the diabetogenic action of anterior pituitary extracts. The criterion of a diabetogenic action adopted has been the induction of hyperglycaemia, glycosuria, ketonuria and polyuria after 3–4 days' daily injection of the extract.

Methods

Estimations. Urine was collected under toluene from metabolism cages. Urinary sugar was determined by Benedict's method, the reagent being standardized against pure glucose. Urinary ketone bodies were assessed semiquantitatively on the basis of the Rothera and Gerhardt tests, and the results expressed in units on an arbitrary scale. As shown by estimation of ketone bodies in urine by the Van Slyke-Denigès method, 5 units on the scale are approximately equal to 1 g. of "total acetone". Blood sugar was estimated by a slight modification of the Hagedorn and Jensen method.

Administration of extracts. Both the intraperitoneal and subcutaneous routes have been used in these experiments, but the former method of administration seems definitely superior to the latter in the case of the dog, so that in most experiments on this animal this route has been used. The skin is prepared for injection by thorough sterilization with soap and water and alcohol, or mercuric chloride, and a sterile needle is used for injection, which is preferably made through the midline. If such simple conditions are observed a long series of intraperitoneal injections of crude extracts may be made into dogs without the development of the slightest symptoms of infection or ill health; indeed, in many cases, the animal appears to thrive on the injections, in spite of the fact that a large amount of crude extract may be administered daily. Occasionally in dogs mild vomiting follows the first few injections, but usually this phase quickly passes off and later injections occasion no untoward symptoms. In a few cases a superficial skin infection developed in injected dogs, but this usually cleared up without treatment. Three cases of peritonitis, following accidental infection, have occurred in a series of over forty injected dogs; these animals were killed immediately infection was diagnosed and the results from these experiments are not included in the data presented. The cat resembles the dog in reacting better to intraperitoneal injection, but with the other species used the subcutaneous route seems preferable.

Care of animals. In these experiments the proper care of animals is vitally important. Houssay [1936] has pointed out that fasting causes the diabetic condition engendered by anterior pituitary extracts to disappear. In the present experiments it has become increasingly clear that unless the experimental animal is in good health and has a normal appetite, results are unsatisfactory. As dogs have been used for long periods in many of the experiments, it has been considered advisable to allow the animals adequate exercise outside the metabolism cages with consequent risk of urine loss. Training of the animals has ensured that the losses have been minimal, in some cases negligible, but it is necessary to point out that the figures given for sugar excretion in this paper are minimum values. Young (6–18 months) male dogs have been used whenever possible, but sometimes older animals and bitches have had to be used, although they are generally less satisfactory than young male animals. In all cases adequate time has been allowed for the animals to become accustomed to the diet and surroundings before injections have begun. All animals were provided with food and water *ad lib.* and in many instances the amount of each constituent consumed was determined by weighing. Dogs received a mixture of cooked meat, raw meat, cooked liver and biscuits, and bones, to which were occasionally added cooked fish and milk. Rabbits were given cabbage or carrots with oats

and bran, while cats received milk, fish and cooked meat. The other species used received appropriate mixed diets.

Preparation of extracts. Unless otherwise stated all extracts were prepared from ox pituitary glands removed from the carcass at the slaughter-house within an hour of the death of the animal. Immediately after the glands had been removed from the animal they were frozen in solid carbon dioxide and transported to the laboratory in this state. The glands were dissected in a semi-frozen condition, a clean separation being effected between posterior and anterior lobes. For "crude saline extracts" the anterior lobes were minced while still frozen and ground in the cold store with sand and 2 ml. cold saline/g. of fresh tissue. After 1 hr. at 0° the mixture was centrifuged in chilled pots at about 1500 r.p.m. for 5 min., and the thick supernatant fluid poured off and stored at 0° without freezing. The residue was discarded. The extract was always used within 5 days of preparation.

An alkaline extract of fresh gland was prepared by grinding the fresh frozen anterior lobes in the cold store with cold saline at pH 8.5 (faint blue tint with thymol blue) in the proportion of 3 ml./g. of fresh tissue, the pH being adjusted and maintained by the frequent addition of small amounts of *N*/5 NaOH. The extraction was allowed to proceed overnight in the cold store, and next morning the residue was centrifuged off and discarded. An acid extract was prepared similarly by extraction with cold saline at pH 2.5. The pH of the extract was adjusted to 8.5 before injection. Alkaline and acid extracts of fresh gland were stored at 0° for not longer than 5 days before injection.

In the preparation of extracts of fresh gland the residue remaining after one extraction with neutral, acid or alkaline saline has always been discarded. If *X* g. of anterior lobe tissue were used, the extract obtained is described as "an extract of *X* g. of fresh gland", abbreviated for convenience to "*X* g. equivalent of extract". It is clear that such an amount of extract does not contain all the extractable material in *X* g. of gland, owing to the undetermined loss in the discarded residue. If one assumes that anterior lobe tissue contains 80% water, then the loss of extractable material by the above method of extraction is about 30% of the total.

Acetone-dried gland was prepared by mincing the dissected gland into 20 vol. of ice-cold dry acetone, and filtering and drying the dehydrated tissue after 24 hr. extraction. The desiccated gland was stored at 0° *in vacuo*; in this condition the diabetogenic activity was retained for at least 1 week. This material was extracted by stirring at room temperature with 7.5 ml. of saline/g. at pH 8.5 or 2.5 for 1 hr., the pH being maintained by the frequent addition of small amounts of *N*/5 NaOH or HCl; the extraction was repeated after centrifuging and the centrifuged extracts combined, that made by acid being adjusted to pH 8 before injection. The acid or alkaline extracts of acetone-dried gland were prepared freshly each day immediately before injection.

Results

Although the blood sugar of injected animals has been determined at intervals, more attention has been paid to the daily sugar excretion. The extent of the daily glycosuria is a more satisfactory indication of the defective metabolism of sugar over 24 hr. than are isolated blood sugar estimations. Determination of the blood sugar has always shown glycosuria to be accompanied by marked hyperglycaemia.

Experiments with mice, rats and guinea-pigs. Numerous experiments on the injection of a crude saline extract into mice, rats and guinea-pigs have yielded

unsatisfactory results. Rats received 1 g. equivalent of crude saline extract subcutaneously each day. About 10% of these exhibited slight glycosuria (0.5% of sugar), but comparable results were obtained with control animals receiving a similar extract of fresh rabbit liver. Groups of 6 rats injected daily for periods up to 3 weeks showed no significant rise in mean blood sugar level [cf. Richardson & Young, 1937]. Similar results were obtained with mice. Guinea-pigs exhibited ketonuria when a crude extract was administered subcutaneously (2 g. equivalent/day of crude extract), but glycosuria did not appear. In all three species the urine excretion and food intake of the injected animals were usually subnormal. It was clear that animals of these species would not serve satisfactorily as test animals for the consistent demonstration of diabetogenic activity.

Experiments with rabbits. In view of the striking results of Baumann & Marine [1932], who were able to induce a glycosuria of as much as 35 g./day in a rabbit by the daily administration of a small amount of crude gland extract, the results recorded here are very disappointing. In a series of experiments in which crude saline extract (1–3 g. equivalent each day) was administered subcutaneously or intraperitoneally for long periods to nearly 100 rabbits, the maximum sugar excretion encountered was 13.5 g./day, while about 25% of the animals did not excrete sugar at all. No difference in reaction was observed between young animals and adults, or between does and bucks; nor was any difference found between the following strains: Dutch, Himalayan, Belgian hares and Sandy lop-eared. In contrast to the results of Baumann & Marine [1932] polyuria did not result from the administration of crude extracts to rabbits; in most instances a diminution of urine volume was observed, and even in those animals in which definite glycosuria occurred no marked polyuria was exhibited. Ketonuria frequently occurred, but was never very striking.

Baumann & Marine do not state by what route their extract was administered to rabbits. In our experiments both the subcutaneous and intraperitoneal methods of injection were used, but in both instances the injected animals appeared to suffer from some degree of shock with consequent diminution of food intake. In control experiments in which a saline extract of fresh tissue other than the pituitary gland was injected into rabbits glycosuria (max. 1.8 g./day) occurred in 2 of 8 injected animals.

Our results with rabbits are the more surprising in that these animals exhibit marked resistance to the hypoglycaemic action of insulin after administration of anterior pituitary extracts [Cope & Marks, 1934; Young, 1936].

It is clear from the foregoing results that the rabbit, although more satisfactory than the mouse, rat or guinea-pig, is not entirely suitable as a test animal for the exhibition of diabetogenic activity.

Experiments with cats. In a series of 8 experiments in which 2–6 g. equivalent of crude saline extract were injected intraperitoneally or subcutaneously into cats each day, four instances of glycosuria were observed, the maximum sugar excretion being 6.4 g./day. The urine volume was usually slightly increased though marked polyuria was absent. Ketonuria was slight. Cats did not take kindly to the experimental conditions or to the administration of extract, and were not considered suitable as test animals.

Experiments with dogs. The results of experiments with dogs are in welcome contrast with those of other species. Of 25 animals injected with suitable extracts, only one failed to exhibit glycosuria, ketonuria and polyuria. In most instances the diabetic symptoms were of remarkable intensity, a glycosuria of 10 g./kg. body weight/day frequently being recorded.

(a) *Injection of crude saline extracts of fresh gland.* In this group the only negative result in 12 experiments was that with a pregnant bitch, which failed to respond to a preparation of extract which exhibited striking diabetogenic activity on injection into a normal male animal. The results of a typical experiment are given in Fig. 1. The figures on the arrows give the weight of fresh

Fig. 1.

Fig. 2.

Fig. 1. Dog. The figures on the arrows give the weight, in g., of fresh ox anterior lobe used to prepare the amount of extract injected daily, from the day indicated by the arrow, onwards. Daily injections ceased on the day indicated by the arrow carrying 0.

Fig. 2. Dog. The figures on the arrows give the weight, in g., of fresh ox anterior lobe used to prepare the amount of extract injected daily, from the day indicated by the arrow, onwards. Daily injections ceased on the day indicated by the arrow carrying 0.

anterior lobe in g. used to prepare the amount of extract injected daily, from the day indicated by the arrow, onwards. The daily intraperitoneal administration of 10 g. equivalent of saline extract was begun on the first day of experiment, after an initial control period. Polyuria and ketonuria appeared on the 3rd day but the excretion of sugar did not begin until the 5th day of injection when the daily pituitary injection was increased to 15 g. equivalent of extract. During the daily injection of 15 g. equivalent of extract the glycosuria (Fig. 1) increased until the 7th day of injection after which it began to decrease, finally disappearing on the 11th day, at which time the urine had become free from ketone bodies. It is interesting to note that at this time the daily urine volume was still much above the pre-injection level. The phenomenon of the disappearance of the symptoms of diabetes despite the continued daily administration of that dose of extract which induced the appearance of diabetes, has occurred in all animals showing a diabetic response, including species other than the dog. If the daily administration of 15 g. equivalent of extract (Fig. 1) had continued from the 11th day onwards it seems most improbable that glycosuria would have reappeared. Nevertheless when the daily dose was increased to 20 g. equivalent, on the 11th day glycosuria and ketonuria reappeared, to fall again to zero on the 16th day of injection.

It was clearly of interest to determine if a daily level of injection could be reached which was of sufficient magnitude to prevent the animal becoming resistant to the diabetogenic action of the extract. With this object the daily dose was increased to 25 g./day on the 16th day of injection. This resulted in

the reappearance of glycosuria which rose to 130 g./day on the 21st day, thereafter declining to about 50 g./day without showing any tendency to diminish further. On the 24th day the ketonuria reached a high level, and on the 26th day the animal passed into a semi-comatose condition. At this time the CO_2 -combining power of the blood was 29 vol. % compared with the normal value for the dog of 40 vol. %. Injections were stopped on this day and the ketonuria subsequently rapidly decreased, but the glycosuria persisted, and, 4 weeks after injections had ceased, reached 264 g./day. The subsequent history of this animal has been described elsewhere [Young, 1937, 2].

For the purpose of detection and possible assay of the diabetogenic activity of the extract it was obviously of advantage to determine conditions in which the symptoms of diabetes were present continuously. With this object, a dog (Fig. 2) received 3 daily injections of 5 g. equivalent followed by 3 daily injections of 10 g. The daily injection was increased to 15 g. on the 7th day, to 20 g. on the 10th day and to 25 g. on the 13th day. Injections ceased on the 16th day. By thus rapidly increasing the dose the animal was induced to remain continuously diabetic. This animal remained diabetic after injections were stopped and his subsequent history will be described elsewhere.

(b) *Injection of alkaline or acid extracts of fresh gland.* Experiments with acid or alkaline extracts of fresh gland gave results similar to those for saline extracts, and need not be described here. Extracts prepared in this way appeared to be no more potent than those obtained by neutral saline extraction.

(c) *Injection of alkaline or acid extracts of acetone-desiccated gland.* Acid or alkaline extracts prepared from dried gland appear to be somewhat less potent than those obtained from fresh gland, but nevertheless were able to induce the appearance of the symptoms of diabetes. In Fig. 3 are shown the results of a

Fig. 3. Dog. The figures on the arrows give the weight, in g., of acetone-desiccated ox anterior lobe used to prepare the amount of extract injected daily, from the day indicated by the arrow, onwards. The arrow carrying *A* indicates the substitution, for a short period, of an acid extract for an alkaline one. The arrow carrying *P* indicates the day on which the dog was depancreatized. The dog died on the 64th day of the experiment.

typical experiment with an alkaline extract of acetone-desiccated gland. The figures on the arrows indicate the weight of desiccated gland (approximately 20% of the weight of the fresh gland) in g. used to prepare the amount of extract injected daily. From the 24th to the 28th days inclusive an acid extract (of 4 g. of desiccated gland) was injected daily instead of an alkaline one; the lack of

response to this substitution, together with the reappearance of glycosuria when an alkaline extract of 5 g. of desiccated gland was administered, indicates that the acid extract was probably no more potent than the alkaline preparation. On the 45th day after pituitary injections were begun, this dog was anaesthetized and the pancreas was removed aseptically. He made a rapid recovery from the operation, but began to lose weight rapidly and died 21 days after pancreatectomy. This rapid loss of body weight is surprising inasmuch as the glycosuria and ketonuria were relatively small, and the animal's appetite good. The completeness of the pancreatectomy was verified at autopsy when the liver fat content was found to be 31 %.

When the daily dose of an alkaline extract of desiccated gland was rapidly increased (Fig. 4) the diabetic condition disappeared but subsequently returned.

Fig. 4. Dog. The figures on the arrows give the weight, in g., of acetone-desiccated ox anterior lobe used to prepare the amount of extract injected daily, from the day indicated by the arrow, onwards. The dog was killed on the 18th day of the experiment.

This animal was killed on the 18th day of injection at which time the sugar excretion was 48 g./day. The liver glycogen content was found to be 1.4 % and the liver fat 7.8 %.

(d) *Control experiments with dogs.* As the diabetogenic action of anterior pituitary preparations is shown in normal animals only after the injection of a large amount of crude extract, often into the abdomen, it was desirable to carry out rigid control experiments. Those performed fall into two classes: (1) those with non-diabetogenic anterior pituitary preparations; (2) those with a crude extract of tissues other than the anterior pituitary gland.

Experiments in the first class were made with extracts of commercial dried anterior lobe or with extracts of fresh gland that had been kept at room temperature for 12 hr. or more. The methods of extraction used for these control experiments were similar to those used for the preparation of active extracts, and the volume of extract injected was similar to that used in previous experiments. In no case was glycosuria or ketonuria induced, the only observed effect of the injections being a striking rise in daily urine volume, in some cases to 2–3 l. per day. This diuretic action of anterior pituitary extracts has been previously observed by other workers [Teel, 1929; Barnes *et al.* 1933]. In the present investigations polyuria without glycosuria was induced by anterior pituitary injections only in cats and dogs. In the other species investigated the urine excretion showed a tendency to fall rather than to rise.

The intraperitoneal injection of crude extracts of rabbit muscle or liver apparently had no effect whatever on our dogs. In Table I is shown the result of an experiment in which an extract of rabbit muscle was injected intraperitoneally into a dog. It will be seen that such an extract has no diuretic action, and had no visible effect on the animal whatever.

Table I. *Daily intraperitoneal injection of a crude extract of rabbit muscle into a dog*

Day	Daily urine vol. ml.	Urinary sugar	Urinary ketone	Amount of extract injected daily (g. of fresh tissue)	Wt. kg.
Control period	Av. = 200	0	0	0	9.40
1	270	0	0	10	—
2	365	0	0	10	—
3	135	0	0	10	—
4	175	0	0	15	—
5	275	0	0	15	—
6	200	0	0	15	—
7	280	0	0	20	—
8	190	0	0	20	—
9	310	0	0	20	10.20

Such control experiments suggest that the diabetogenic action of a crude anterior lobe extract cannot be ascribed to a non-specific action of a voluminous intraperitoneal injection of crude protein solution.

DISCUSSION

If the criteria of the existence of a diabetic condition are considered to be the exhibition of hyperglycaemia, glycosuria, ketonuria, polyuria and polydipsia, then of the species examined in the present investigation only the dog (and possibly the cat) developed diabetes in response to the anterior pituitary injections. In the other species, polyuria, together with polydipsia and polyphagia were usually absent. It is remarkable that dogs exhibiting a profound glycosuria have lost little or no weight in the present experiments, differing in this respect from depancreatized animals and from many clinical cases of diabetes mellitus.

The relative insensitivity of species other than the dog to the diabetogenic action of crude anterior lobe extracts may account for many failures to confirm Houssay's original finding. The present investigation certainly provides strong support for Houssay's contention that the anterior pituitary lobe contains a diabetogenic substance effective in intact animals. The reason for the marked species difference is not clear. It is interesting that the two species found to give the best results (cat and dog) are both carnivorous, although no great significance can be attached to this observation at present. It may be emphasized here that the dogs used in this investigation have received a normal, high protein diet, and it has proved unnecessary to provide a high proportion of carbohydrate food in order to make the effects of the pituitary extracts manifest. Our failure to confirm the results of Baumann & Marine with rabbits is puzzling, and there appears to be no simple explanation at present. It seems improbable that a difference in the type of rabbit used can account for such a marked difference in results. Furthermore, a number of different strains of rabbit have been found, in the present investigations, to give similar results when injected with extract.

Houssay has repeatedly stressed the importance of using fresh glands for the preparation of diabetogenic extracts, and of preparing and storing the extract at

low temperature. In the present investigation these points have been found to be of paramount importance, and it seems that unless there is available a supply of fresh glands frozen immediately after removal from the animal, satisfactory results cannot be expected. It is clear, however, from the present investigation, that the diabetogenic activity of fresh gland is not lost when the tissue is desiccated in cold acetone and stored for 1 week *in vacuo* at 0°.

The disappearance of the symptoms of diabetes in dogs and other animals despite the continued daily administration of a fixed amount of extract, together with their reappearance when the daily dose is increased, is of great interest. The production of an "antihormone" of the type postulated by Collip [1934] seems improbable because of the rapidity of the reaction. Pancreatic tissue removed from a dog which had become resistant to the diabetogenic action of the extract after displaying an initial response was examined histologically by Dr K. C. Richardson, who reported unusual mitotic activity in the islet cells. It therefore seems possible that the animals are able to develop a resistance to the diabetogenic action of the extract because the pancreatic islet tissue becomes hypertrophic, with consequently enhanced insulin secretion. The question of the action of anterior pituitary extracts on pancreatic islets has been further investigated and is the subject of another communication [Richardson & Young, 1937]. If the diabetogenic factor in anterior pituitary extracts is accompanied by a more slowly acting "pancreatotropic substance" acting antagonistically via the pancreas, then the results with dogs might receive a rational explanation. Moreover, the relative ineffectiveness of diabetogenic extracts in other species might result from their rapid and effective response to the pancreatotropic substance. Such a suggested explanation does not require the assumption that the diabetogenic factor or the pancreatotropic substance are separate entities, nor does it entail that the diabetogenic factor should act only by an extra-pancreatic mechanism.

Collip [1935] has described an experiment in which a depancreatized dog, maintained on insulin, was treated with an anterior pituitary extract rich in both growth and ketogenic principles, for a period of 4 months, after which insulin therapy was stopped. For a period of 1 month thereafter the animal showed no increase in acetonuria, although marked hyperglycaemia and glycosuria were present. In an attempt to obtain similar results in the present investigation the animal, whose curves are given in Fig. 3, was depancreatized 42 days after daily injections of crude anterior pituitary extract had begun. Although ketonuria had been absent for 21 days before pancreatectomy, i.e. the animal had become resistant to the ketogenic action of the anterior pituitary extract, a substantial ketonuria appeared after removal of the pancreas. The animal rapidly lost weight and died 21 days after pancreatectomy, giving no evidence of the presence in its blood of an antihormone to the diabetogenic or ketogenic substances of the anterior pituitary gland.

Houssay [1936] distinguishes two types of hyperglycaemic action of anterior lobe extracts, one, an immediate blood sugar-raising action and the other—the true diabetogenic action—exhibited only after repeated daily injections. As Serio [1936] and Houssay [1937] have shown, the daily administration of adrenaline, which has a marked and immediate hyperglycaemic effect, does not produce a diabetic condition in dogs. The results of the present investigations are in entire agreement with Houssay's views. The administration of the diabetogenic extracts (1 g. equivalent/kg. body wt.) used in the present experiments has no marked immediate effect on the blood sugar level of the injected animal, the general tendency being a slight fall of about 10 mg./100 ml. during 2–3 hr.

following injection. Nevertheless, if such a fresh pituitary extract is allowed to remain at room temperature for several hours, it loses its true diabetogenic activity and may simultaneously acquire the ability to cause hyperglycaemia immediately after injection. One is perhaps not justified in saying on this basis alone that the immediate blood sugar-raising action of pituitary extracts is of no physiological significance, although such may well be the case. The injection of a fresh, crude extract of posterior pituitary lobe can cause an immediate rise of blood sugar level, and contamination of anterior lobe extract with posterior lobe principles may account for some of the published results in which anterior lobe extracts were found to exert a rapid hyperglycaemic action.

It seems necessary again to emphasize the need for greater precision in the use of the term "diabetogenic". This description should be applied only to those extracts which possess what Houssay describes as a "true diabetogenic activity", i.e. their injection has little or no immediate effect on the blood sugar level, although continued daily administration induces, after a latent period of some days, a state of hyperglycaemia, glycosuria, ketonuria, polyuria and polydipsia. The active principle or principles concerned should be described as the "diabetogenic factor" and not as the "diabetogenic hormone". Investigations in which the injection of an anterior pituitary extract is shown to induce nothing more than a rise of blood sugar level within a few hours of injection, should not be described as demonstrating a "diabetogenic action" of the pituitary preparation. Extracts having nothing more than this type of effect on the blood sugar level should be described as "hyperglycaemic" and not "diabetogenic". This discussion is relevant to a recent paper of Elmer *et al.* [1937]. These authors injected *intravenously* into rabbits an acid extract of 1 g./kg. body wt. of dried anterior lobe (i.e. weight for weight 5 times the initial dose used in the present investigation), finding a rise of blood sugar varying between 250 and 30 mg./100 ml. during the following 3 hr. No control experiments using tissue extracts other than anterior pituitary preparations are described. Despite the fact that the recognized symptoms of diabetes mellitus include, beside hyperglycaemia, glycosuria, ketonuria, polyuria and polydipsia, and despite Houssay's views, quoted above, these results are described as a demonstration of the diabetogenic action of anterior lobe extract, in agreement with Houssay's findings. On this basis, extracts of the adrenal medulla and of the posterior pituitary lobe contain far more diabetogenic factor than those of the anterior pituitary gland, while a partially autolysed non-specific tissue extract containing peptone might be judged as an especially rich source.

One may conclude, however, from the results of the present investigation that the normal male dog is an excellent test animal for the examination of the true diabetogenic action, in intact animals, of anterior pituitary extracts. The application of this finding to preliminary attempts to fractionate diabetogenic extracts is described in the following paper.

SUMMARY

1. The diabetogenic action of crude ox anterior lobe extracts has been investigated in the following species: mouse, rat, guinea-pig, rabbit, cat and dog.
2. The mouse, rat and guinea-pig appear to be almost completely insensitive to the diabetogenic action of an extract which is effective in other species. With rabbits and cats, glycosuria and ketonuria followed the daily injection of a crude extract in 25-50 % of the cases investigated, but with dogs, only one failure (in a pregnant bitch) was recorded in 25 experiments.

3. In the dog, glycosuria, hyperglycaemia and ketonuria appear only after 3 or 4 daily injections of extract, and may then disappear within 4 or 5 days in spite of the continued daily injection of the same dose of extract. The diabetic symptoms reappear, however, if the daily dose is suitably increased, and may again subside, only to be revived by a further increase in the amount of extract injected daily. If a sufficiently large daily injection be given the diabetic condition may continue indefinitely after cessation of injections [Young, 1937, 2].

4. Preparation and conservation of the extract at low temperature are essential for the demonstration of diabetogenic activity. Acetone-desiccation of the fresh gland appears to diminish, without entirely destroying, its diabetogenic activity.

5. With dogs, a non-glycosuric polyuria (up to 300 ml./kg./day) may be induced by the daily injection of anterior lobe extracts of which the diabetogenic factor has been inactivated. Such a polyuric action of crude extracts was not observed in mice, rats, guinea-pigs or rabbits.

6. The diabetogenic extracts used in the present investigation do not cause any marked rise of blood sugar immediately after injection. The importance is stressed of differentiating clearly between a true diabetogenic action exhibited only after 3 or 4 daily injections of extract, and the action of other types of extract in causing a rise of blood sugar level within a few hours of administration. The name "diabetogenic factor" (not hormone) should be reserved for the substance (or substances) present in anterior pituitary extracts, which possesses true diabetogenic activity and has little or no action on the blood sugar level within a few hours of injection.

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