BRITISH MEDICAL JOURNAL

LONDON SATURDAY DECEMBER 2 1944

"GROWTH" AND THE DIABETOGENIC ACTION OF ANTERIOR PITUITARY PREPARATIONS

II. GROWTH AND EXPERIMENTAL INSULIN-INSENSITIVE DIABETES

BY

F. G. YOUNG, D.Sc., Ph.D.

(From the National Institute for Medical Research, London, and St. Thomas's Hospital Medical School)

In the previous paper in this series (Young, 1941) it was shown that, when treated daily with a dose of crude anterior pituitary extract greatly in excess of that required to produce diabetes in adult dogs, puppies respond with an increase in growth rate and fail to exhibit any symptoms of diabetes. It was then reported that when one puppy had been treated daily for nearly five months he ultimately became intensely diabetic, and then stopped growing despite continuation of pituitary treatment.

The present paper describes investigations into the relationship between growth and the diabetic condition of this and other similar animals.

Methods

Animals.—Normal adult dogs, normal puppies, and adult dogs which had been rendered permanently diabetic by a short period of treatment with anterior pituitary extract (Young, 1937) were used. They were weighed daily and the urine excreted over 24-hour periods collected. Their diet was meat and vitamin concentrates, and, unless otherwise indicated, they were allowed to eat *ad libitum*, the amount of food consumed being determined daily.

Anterior Pituitary Extract.—This was the Seitz-filtered crude alkaline extract of fresh ox anterior pituitary lobe previously described (Young, 1941). The concentration was such that 1 ml. contained the material extracted from 250 mg. of fresh anterior lobe tissue. It was injected subcutaneously.

Estimations.—Urinary sugar was determined by Benedict's method, and urinary ketones by the method of van Slyke. The nitrogen contents of food, urine, and faeces were all estimated by the Kjeldahl method. Blood sugar was determined by the method of Hagedorn and Jensen.

Insulin Sensitivity.—The available carbohydrate of the food consumed was computed from the formula $G + 0.58P + 0.1F$, where G = preformed carbohydrate, P = protein, and F = fat content of the diet. The figure for the daily intake of available carbohydrate minus the amount of glucose excreted in the corresponding 24-hour period gave the amount of carbohydrate utilized. When the diabetic condition of the animals had been controlled, or almost controlled, by the daily subcutaneous administration of insulin, the available carbohydrate utilized was divided by the dosage of insulin given. In our experiments this quotient (g. of available carbohydrate utilized per unit of insulin administered) has not differed significantly if two doses of ordinary insulin are given with two separate meals, or one dose of P.Z.I. administered and the animals allowed to consume the food over the whole 24-hour period. We have taken this quotient as an index of the insulin sensitivity of the diabetic animal (cf. Falta, 1936), and, although it may vary from animal to animal, it is usually substantially constant from time to time in any given diabetic dog (cf. Marks and Young, 1939).

Results of Prolonged Treatment of Puppies with Diabetogenic Extract

Five puppies were treated daily with the crude extract for a period of 100 days or more, and in every case they grew about twice as fast as control animals. Dogs 124 and 126 received a brief period of treatment with doses of extract which rose from 5 ml. to 20 ml./day during the first four weeks of the experiment and then fell to and remained at 5 ml./day. The other animals received 5 ml./day throughout the experiment. A brief summary of the history of these animals is as follows:

Dog 124 (male black-and-tan terrier; initial weight 2.44 kg.) became diabetic after 131 days of treatment (weight 20.20 kg.), but the diabetic condition disappeared shortly after pituitary treatment ceased. Details of this animal's history are given later in this paper.

Dog 125 (male cross-spaniel; initial weight 1.73 kg.) became diabetic after 239 days of treatment (weight 13.40 kg.), and the diabetic condition persisted after pituitary treatment ceased.

Dog 126 (female fox-terrier; initial weight 3.71 kg.) developed an enlarged thyroid gland after about 80 days of treatment (weight 14.15 kg.). The enlargement persisted until the animal was killed, without having developed diabetes, after 329 days of treatment (weight 29.10 kg.).

Dog 134 (male black-and-tan terrier; initial weight 1.87 kg.) became mildly diabetic after 126 days of treatment (weight 15.40 kg.), but continued to grow for a time, and then became sugar-free again for a brief period. But a return of glycosuria, with a rise to nearly 50 g./day on day 157 (weight 19.70 kg.), led to cessation of growth, and the animal was killed on day 166, daily injections of extract being still in progress (weight 19.30 kg.).

Dog 137 (female black-and-tan terrier; initial weight 2.34 kg.) died in her sleep from no obvious cause after 119 days of treatment; a diabetic condition had not then developed. When she died this animal weighed 15.80 kg. at a time when her litter-mate control male weighed 8.85 kg.

Thus it will be seen that of the four dogs surviving 150 days of treatment three ultimately became diabetic, the date of appearance of diabetic symptoms varying between 126 and 239 days. The puppies were all 2 to 3 months of age at the beginning of the experiment, and by the time they had become responsive to the diabetogenic action of that dose of pituitary extract which initially exerted a growth-promoting and not a diabetogenic action they were 6 months of age or more—i.e., they had become dogs rather than puppies. All three animals which became diabetic ceased growing after severe diabetes had developed; but dog 126, which developed an enlarged thyroid gland early in the experiment and did not become diabetic even after pituitary treatment lasting nearly 11 months, continued to grow throughout the experiment. Although the goitre subsided somewhat towards the end, at post-mortem examination the thyroid gland was found to be 10 to 20 times the size of that for a normal dog of similar weight, and it is

probable that this dog persistently suffered some degree of thyroid deficiency after the goitre had developed. It is possibly relevant that thyroidectomy diminishes somewhat the intensity of pancreatic diabetes (Dohan and Lukens, 1938), and of the diabetogenic action of anterior pituitary extract in the hypophysectomized-depancreatized toad (Houssay, Biasotti, and Rietti, 1934).

Histology of the Pancreatic Islets in the Long-treated Puppies

Dr. H. Hughes, of the Department of Anatomy, St. Thomas's Hospital Medical School, kindly examined histologically the pancreatic material from these animals, and his report will be published in full elsewhere. With his permission the following summary of his findings is given.

Dog 124.—Pancreas not grossly abnormal, though many β cells contained only a few specific cytoplasmic granules.

Dog 125.—Gross destruction of β cells, with some reduction in the number of α cells. Vacuolation of duct epithelium marked (cf. Richardson, 1940).

Dog 126.—Substantial increase in the number of β cells, some islets consisting solely of such cells. No vacuolation of duct epithelium observed.

Dog 134.—Intense hydropsis of β cells observed, with apparent increase in number of islets and total amount of islet tissue. The number of α cells was possibly abnormally low.

The histological findings are in general agreement with the metabolic observations.

Influence on Growth of Simultaneous Treatment with Diabetogenic Pituitary Extract and Insulin

The results of the above long-term experiments clearly supported the conclusion drawn in the previous paper that "an increase in body weight . . . can be regarded as neutralizing, for a time at least, the diabetogenic influence of the anterior pituitary gland" (Young, 1941). An interesting possibility now arose with respect to the grown "puppy" that had become responsive to the diabetogenic influence of pituitary treatment, and had then ceased to grow despite continuation of the same treatment as had initially stimulated growth without precipitating diabetes. Would such an animal grow if pituitary treatment were accompanied by the administration of sufficient insulin to suppress, or at least depress, the diabetogenic effect of the pituitary extract? Experiments were therefore carried out on these lines.

Dog 124.—Although sugar first appeared in the urine of this animal on the 131st day of the experiment, the rate of growth under the influence of pituitary treatment alone was initially unaltered, remaining at about twice that of control animals (Fig. 1). But when the glycosuria exceeded 30 g./day growth stopped, both body weight and glycosuria then remaining approximately constant for a period

of about 2 weeks. Thereafter the rate of excretion of sugar rapidly rose to about 200 g./day, and the body weight simultaneously began to fall. Cessation of pituitary treatment on day 156 was quickly followed by disappearance of glycosuria, while the body weight remained substantially constant, though subject to some fluctuation. These results illustrate well the reciprocity between the growth-promoting and diabetogenic actions of the crude pituitary extract (Fig. 1). Subsequently, daily pituitary treatment was resumed for a short period (days 188–193), and although the body weight showed an inclination to rise in response to this stimulus, glycosuria, accompanied by an alarming ketonuria, appeared (Fig. 2). On day 210 a single injection of extract induced a slight but definite excretion of

FIG. 2.—Data for dog 124 over period 24–37 weeks. The points indicated by the arrows are:—3: Daily injection of pituitary extract reinstituted. 4: Daily injection of extract stopped. 5: Single injection of extract given. 6: Daily injection of pituitary extract and insulin given from this point onwards.

sugar, which suggested that the animal was in a pre-diabetic condition. On day 224 the daily administration of 5 ml. of the pituitary extract was again begun, protamine-zinc-insulin now being simultaneously injected to control the diabetes thus induced. The initial daily dose of insulin was 20 units, but a dangerous degree of ketonuria developed, the daily excretion of ketone (equivalent acetone) rising above 10 g. despite rapid elevation of the daily dose of insulin to 120 units (Fig. 2). Ketonuria was almost entirely suppressed by raising the insulin dosage to 400 units/day, but even 2,000 units a day did not completely control the glycosuria (Fig. 2). Nevertheless, as the result of this simultaneous treatment with pituitary extract and insulin, the dog rose in body weight by nearly 7 kg. (Fig. 2 and Table I). It should be pointed out that the amount of food eaten by this animal was not controlled, and that he ate more as the result of the treatment with pituitary extract and insulin (Table I). During the last few weeks of the experiments samples of blood plasma from this dog were tested for anti-insulin activity in the rabbit (de Wesselow and Griffiths, 1936), with negative results.

Dog 125.—Unlike dog 124, this animal became persistently diabetic as the result of prolonged treatment (239 days) with pituitary extract alone. An experiment similar to that carried out on dog 124 was then performed on this animal, with the main difference that the food intake was controlled and fixed at 894 g. cal./day throughout the experiment and that the animal received a small daily dose of insulin before pituitary treatment began (Table I). This animal also increased in body weight as the result of simultaneous treatment with insulin and pituitary extract, the mean daily dose of insulin required to control the diabetic condition during pituitary treatment being 330 units, compared with 20 units/day for the period without simultaneous pituitary treatment. This experiment showed that the insulin sensitivity of the diabetes induced by the pituitary treatment was still low even when the food intake was restricted to a constant level, and, furthermore, that the growth-promoting action of simultaneous treatment with pituitary extract and insulin could be demonstrated under such conditions.

Dogs 115 and 132.—These were two adult (female and male respectively) dogs which had been rendered permanently diabetic by a short course of pituitary extract. Dog 115 had been diabetic for nearly 2½ years, and dog 132 for nearly 1 year, when the experiments described here were carried out. Both animals were apparently adult at this time. Two experiments involving the simultaneous administration of insulin and pituitary extract were carried out with dog 132; in one of these the animal was allowed

FIG. 1.—Data relating to dog 124, treated daily with diabetogenic pituitary extract. (a) Growth curve for whole experiment. The data plotted are the mean weights for periods of one week. The control curve is based on data for four untreated animals of the same breed as dog 124. (b) Sugar excretion over weeks 18–25. (c) Daily variations in weight over weeks 18–25.

The points indicated by the arrows are as follows:—1: Excretion of sugar first observed. 2: Daily injection of pituitary extract stopped. 3: Daily injection of extract resumed. 4: Daily injection of extract again stopped. 5: Single injection of extract given. 6: Daily injection of pituitary extract and insulin given from this point onwards.

to eat 50% more food during the period of simultaneous treatment with pituitary extract and insulin (Table I), while in the other the food consumed was constant throughout. In the single experiment with dog 115 the food intake was constant (Table I). The three experiments on these two animals confirmed the relevant results obtained with dogs 124 and 125—namely, that when the diabetic dogs were treated with pituitary extract and insulin simultaneously they increased in body weight, but that the amount of insulin

often grow normally without exhibiting glycosuria until they have attained a weight of 200 g. or more, after which they may begin to excrete sugar (cf. Foglia, 1944). In his classical studies on the partially depancreatized puppy Langfeldt (1920) found that an operated animal might be free from symptoms of diabetes mellitus for many months, although ultimately, when the animal had grown, the sugar tolerance might decline

TABLE I.—*The Influence in the Diabetic Dog of Simultaneous Treatment with Insulin and Anterior Pituitary Extract*

Dog		Control Period							Experimental Period (5 ml. of Pituitary Extract Injected Daily)							
No.	Condition	Duration (days)	Energy Intake (kg. cal./day)	Energy Utilized		Insulin Administered (units/day)	Available Carbohydrate Utilized (g./unit of Insulin)	Increase in Body Weight (g./kg./day)	Duration (days)	Energy Intake (kg. cal./day)	Energy Utilized		Insulin Administered (units/day)	Available Carbohydrate Utilized (g./unit of Insulin)	Increase in Body Weight (g./kg./day)	Net Increase in Body Weight (Experimental Minus Control) (g./kg./day)
				(kg. cal./day)	(kg. cal./sq. m./day)*						(kg. cal./day)	(kg. cal./sq. m./day)*				
124	Pre-diabetic	21	1,061	1,061	1,260	0	—	0.0	26	2,157	1,680	1,798	400–2,000	0.12	8.6	8.6
132	Diabetic	12	823	740	1,245	25	2.88	0.0	7	1,235	1,059	1,690	285	0.33	21.7	21.7
125		7	894	894	1,440	20	5.30	0.0	11	894	894	1,365	330	0.30	5.5	5.5
132		16	823	762	1,291	30	2.58	1.0	8	823	670	1,105	280	0.20	7.0	6.0
115		12	526	465	963	8.5	5.24	–4.2	4	526	420	865	170	0.19	5.6	9.8

* Body surface in square metres calculated from the formula $0.112\sqrt[3]{W^2}$ (Lusk, 1928), where W = body weight in kg.

required to control the diabetes during the period of pituitary treatment was very large compared with that required in the absence of pituitary treatment (Table I).

It should be mentioned that insulin sensitivity was not, in most instances, constant throughout the experimental period. It fell rapidly after 2 to 3 days of pituitary treatment, and then tended to rise again after 6 to 10 days (cf. Haist *et al.*, 1940). The data given in Table I are the mean values for the stated periods.

Discussion

Growth during Pituitary Treatment.—There is much evidence, direct and indirect, to support the view that pituitary-stimulated growth (together with the accompanying nitrogen retention) is dependent on an increased capacity of the pancreatic islets to secrete insulin. The intact rat does not become diabetic when treated with a pituitary extract which is highly diabetogenic in the adult dog (Young, 1938), and the amounts of islet tissue and of insulin in the pancreas of the rat are both doubled by such treatment (Richardson and Young, 1937; Marks and Young, 1940). Conversely, Marx *et al.* (1943) have recently found that treatment of partially depancreatized rats with a highly purified preparation of growth hormone increases the glycosuria of these animals. Growth is normally accompanied by nitrogen retention, and Mirsky (1939) has presented evidence that the nitrogen-retaining action of anterior pituitary extracts is mediated, at least in part, by the islets of Langerhans of the pancreas, while Gaebler and Robinson (1942) believe that whether or not an anterior pituitary preparation will cause nitrogen storage or exert a diabetogenic effect may depend on the amount of insulin that the pancreas can supply.

In the present experiments it has been shown that during the period of natural growth in the puppy the administration of a pituitary extract which is diabetogenic in the adult animal leads not to diabetes but to an acceleration of growth. When, however, the puppy has reached adulthood under continuous pituitary treatment it may become diabetic and ultimately cease to grow. The treatment of such a diabetic animal, or even of a normal adult diabetic dog, with anterior pituitary extract together with insulin leads again to an increase in body weight, although the daily dose of insulin required to restrain the diabetogenic action of the anterior pituitary extract under these conditions may be very large. For instance, dog 124 required more than 2,000 units/day of P.Z.I. to suppress the diabetogenic action of the daily subcutaneous injection of 5 ml. of the pituitary extract, a dose of extract which during the first 20 weeks of the experiment induced growth uncomplicated by diabetes. It would be unsafe to deduce that during the period of pituitary-stimulated growth the pancreas of the puppy was secreting each day an amount of insulin equivalent in effect to the daily subcutaneous injection of 2,000 units of P.Z.I. It is common experience that rats partially depancreatized at an early age

and a progressive and ultimately fatal diabetes become manifest. The maintenance of normal growth may require less insulin than might otherwise be expected. Nevertheless it seems probable, on the evidence available, that during the period of pituitary-stimulated growth the pancreatic islets of our puppies were secreting more insulin than would have been required for normal growth in the absence of the extra pituitary stimulus. Certainly, once the endogenous insulin supply of our animals proved to be insufficient for further growth the amount of exogenous insulin required for the maintenance of the growth-promoting action of the anterior-pituitary extract was large (Table I). But there is no evidence that at the point when insufficiency of the endogenous insulin supply became manifest the insulin requirement for the control of carbohydrate metabolism remained unchanged; indeed, the requirement may have risen substantially.

It has been assumed in the present paper that the increase in body weight which occurred under the influence of pituitary extract and insulin was indeed representative of true growth. A study of the nitrogen retention during the period of alleged growth and of the composition of the tissues of the enlarged animal will be reported in detail elsewhere, but it may here be stated that the increase in body weight represents, to a large degree, a rise in the amount of protein containing tissue in the animal (Young, 1945). These results serve to emphasize once again the possible significance with respect to clinical diabetes mellitus of the relationship between the growth-stimulating and diabetogenic actions of anterior pituitary secretions.

TABLE II.—*Insulin Sensitivity in Different Types of Insulin-controlled Experimental Diabetes in the Dog*

Condition	No. of Animals	No. of Observations*	Insulin Sensitivity* (g. of Available Carbohydrate Utilized per Unit of Administered Insulin)				Standard Error of Mean	} $t = 2.98$ (Significant difference) $t = 9.13$ (Highly significant difference)
			Maximum	Minimum	Mean			
Diabetes following pancreatectomy	8	20	6.30	1.79	3.73	0.277		
Persistent pituitary diabetes	5	13	5.30	1.71	2.60	0.258		
Persistent pituitary diabetes treated with pituitary extract and insulin	4	5	0.33	0.12	0.23	0.038		

* Each observation is the mean value for a series of daily estimations, over periods of 1 to 4 weeks, at a time when the diabetic condition was under control, or nearly under control, as the result of the administration of insulin.

Insulin-insensitive Diabetes.—The diabetic condition exhibited by our pre-diabetic or pituitary-diabetic animals which are treated daily with diabetogenic anterior pituitary extract is clearly of an insulin-insensitive type. In Table II is given a

summary of the data for insulin sensitivity (grammes of available carbohydrate utilized per unit of insulin administered) for the three types of experimental diabetes we have studied. It will be seen that the previously claimed difference in insulin requirement between the depancreatized and the pituitary-diabetic dog (Marks and Young, 1939) is endorsed, and the very low insulin sensitivity of the diabetic animals, treated with both pituitary extract and insulin, emphasized. Statistical analysis shows that the differences between the insulin sensitivities of the three groups are all significant.

For many years Falta and his pupils have emphasized the differences in insulin sensitivity that are encountered in clinical diabetes mellitus. Falta (1936) divides clinical diabetes into three main classes according to insulin sensitivity: those which are insulin-sensitive and retain 2-3 g. of available carbohydrate for each unit of administered insulin; those of the middle class of insulin sensitivity, who retain 1-2 g./unit; and those whom Falta describes as insulin-resistant and who retain only about 0.1 g. of carbohydrate/unit of insulin injected. The insulin sensitivity of the insulin-resistant class of Falta agrees very well with that of our diabetic dogs treated with both pituitary extract and insulin. Dog 124, which required daily more than 80 units/kg. of insulin for (incomplete) control of the diabetic condition (i.e., 5,600 units for a 70-kg. man), appeared to be at least as insensitive to the action of insulin as the human diabetic usually classed as "insulin-resistant." It is therefore of interest that no "anti-insulin" activity of the plasma of this animal was demonstrable in rabbits, although some well-authenticated instances of a "de Wesselow-Griffiths" effect of blood plasma in human insulin-insensitive diabetes have been recorded (Marble, Fernald, and Smith, 1940; Rushton, 1940). But the fact that dog 124 was receiving such large doses of insulin may have complicated the results of this type of test.

Recently Best *et al.* (1942) have examined the effect of simultaneously administering P.Z.I. and diabetogenic pituitary extract to normal dogs. Their dose of diabetogenic pituitary extract was probably approximately equivalent to ours, and they found that an average of about 40 units of P.Z.I. was required daily to suppress almost completely the diabetogenic action of the anterior pituitary extract. Calculation on the basis of their published data shows that the carbohydrate equivalent of the exogenous insulin in their experiments was 2.49 ± 0.41 g./unit—i.e., the apparent insulin sensitivity of their animals was similar to that for dogs with ordinary pituitary diabetes. This suggests that a large increase in endogenous insulin secretion may occur in the normal dog treated with diabetogenic anterior pituitary extract, and rough calculation indicates that for a 10-kg. dog it may be equivalent to the effect of the daily subcutaneous injection of about 300 units of P.Z.I. As this is probably 10 to 20 times the normal rate of insulin secretion by the pancreas it is perhaps not surprising that degeneration of the pancreatic islets usually follows treatment with diabetogenic pituitary extract in the dog.

Summary

Daily injection of diabetogenic anterior pituitary extract into puppies induced an acceleration of growth uncomplicated by diabetes, which was maintained during treatment lasting four months or more. At varying times after this period three out of four animals became diabetic, and then ceased to grow despite continuation of pituitary treatment. The animal which did not become diabetic had developed an enlarged thyroid gland early in the experiment; this dog continued to grow in response to pituitary treatment lasting nearly 11 months.

Two of the grown puppies in which the growth-stimulating action of the pituitary extract had given way to a diabetogenic one were induced to resume growth when the administration of the pituitary extract was accompanied by the injection of large doses of insulin. Two adult pituitary-diabetic dogs likewise responded to similar treatment by a rise in body weight.

When diabetic or pre-diabetic adult dogs were treated daily with diabetogenic pituitary extract an extremely insulin-insensitive diabetic condition developed. One dog required as much as 80 units/kg./day of body weight for (incomplete) control of this diabetes.

The possible significance with respect to clinical diabetes mellitus of the relationship between the growth-stimulating and diabetogenic actions of anterior pituitary secretions is once again emphasized.

I am indebted to the Medical Research Council for a grant in aid of that part of the work which was carried out at St. Thomas's Hospital Medical School, and to Dr. J. W. Trevan, Director of the Wellcome Physiological Research Laboratories, for a gift of insulin.

REFERENCES

- Best, C. H., Campbell, J., Haist, R. E., and Ham, A. W. (1942). *J. Physiol.*, **101**, 17.
 Dohan, F. C., and Lukens, F. D. W. (1938). *Amer. J. Physiol.*, **122**, 367.
 Falta, W. (1936). *Die Zuckerkrankheit*, Urban and Schwarzenberg, Berlin and Vienna.
 Foglia, V. G. (1944). *Rev. Soc. argent. Biol.*, **20**, 21.
 Gaebler, O. H., and Robinson, A. R. (1942). *Endocrinology*, **30**, 627.
 Haist, R. E., Campbell, J., and Best, C. H. (1940). *New Engl. J. Med.*, **223**, 607.
 Houssay, B. A., Biasotti, A., and Rietti, C. T. (1934). *C. r. Soc. Biol.*, Paris, **115**, 323.
 Langfeldt, E. (1920). *Acta med. scand.*, **53**, 1.
 Lusk, G. (1928). *Science of Nutrition*, 4th ed., Saunders, Philadelphia and London.
 Marble, A., Fernald, A. T., and Smith, R. M. (1940). *Endocrinology*, **28**, 735.
 Marks, H. P., and Young, F. G. (1939). *J. Endocrinol.*, **1**, 470.
 ——— (1940). *Lancet*, **1**, 493; **2**, 710.
 Marx, W., Anderson, E., Fong, C. T. O., and Evans, H. M. (1943). *Proc. Soc. exp. Biol.*, N.Y., **53**, 38.
 Mirsky, I. A. (1939). *Endocrinology*, **25**, 52.
 Richardson, K. C. (1940). *Proc. roy. Soc.*, B, **128**, 153.
 ——— and Young, F. G. (1937). *J. Physiol.*, **91**, 352.
 Rushton, J. G. (1940). *Proc. Mayo Clin.*, **15**, 417.
 Wesselow, O. L. V. de, and Griffiths, W. J. (1936). *Lancet*, **1**, 991.
 Young, F. G. (1937). *Ibid.*, **2**, 372.
 ——— (1938). *Biochem. J.*, **32**, 513.
 ——— (1941). *British Medical Journal*, **2**, 897.
 ——— (1945). (In the press.)

THE ACTION OF PENICILLIN ON LEPTOSPIRA AND ON LEPTOSPIRAL INFECTIONS IN GUINEA-PIGS

BY

J. M. ALSTON, M.B., M.R.C.P.Ed.

AND

J. C. BROOM, M.D.

(From Archway Hospital and the Wellcome Research Institution, London)

The experiments described below were designed to test whether penicillin inhibits the growth of leptospira in culture, and, if so, whether the drug would cure leptospiral infections in guinea-pigs.

In Vitro

Material and Method

Nine strains of *Leptospira icterohaemorrhagiae* were used. Six had been obtained from human infections (Jackson, Hickey, Wijnberg, K.L., Barber, Rachmat); two had been isolated from rats (wild rat and W.P.R.L.); and one from a dog (L 56). The strain of *L. canicola* maintained in the National Collection of Type Cultures was tested also. Some of these strains are virulent for guinea-pigs, and others are completely non-virulent. For the test a solution of penicillin was prepared containing 200 Oxford units per ml., and serial fivefold dilutions were made from it. 0.25-ml. volumes of these dilutions were added to 3 ml. of Fletcher's broth. After mixing, the medium was seeded with 0.25 ml. of a heavy culture of leptospira. The final concentrations of penicillin were thus 50, 10, 2, etc., down to 0.016 Oxford unit in 3.5 ml.

In some later experiments the two highest concentrations were omitted. Most of the tests were carried out with calcium penicillin; in a few parallel experiments crude filtrate of the growth of *Penicillium notatum* in a protein-free medium (Alston, 1944) was used with similar results.

Results

The growth of all these strains was prevented by penicillin in concentrations of 0.4 o.u. and upwards in 3.5 ml. (0.11 o.u. per ml.). With lower concentrations variation of sensitivity to the drug was apparent. Since leptospira multiply more slowly than such bacteria as staphylococci, inhibition of growth compared with controls first becomes noticeable only after about three days' incubation. It was noted that cultures which were inhibited after three days showed no further sign of growth when examined at intervals up to 14 days. Penicillin thus either kills leptospira or maintains its activity against them for one to two weeks at 32° C. It was also found that penicillin cultures, which appeared to be multiplying during the first three days, might show marked diminution in numbers when examined at the end of a week.

This possible lethal, as opposed to inhibitory, effect was examined in another experiment. A well-grown 7-day mass-