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.

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

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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-

culture of leptospira was distributed in 9.5-ml. amounts in test-tubes, and 0.5 ml. of serial dilutions of penicillin, diminishing by 50%, were added. The final series contained 240, 120, down to 0.5 o.u. in 10 ml. The mixtures and controls were incubated at 24° C., and were examined at intervals. As before, counts were made of the number of living motile leptospira in standard microscope fields.

It was found that concentrations of less than 15 o.u. of penicillin in 10 ml. had no apparent effect on the leptospira. After 12 days' incubation the number of living leptospira was greatly diminished in higher concentrations, but the organisms were not entirely eliminated after three weeks' contact with penicillin. No abnormal morphological forms were noted such as occur when staphylococci are acted on by penicillin.

This lethal effect of penicillin on leptospira, associated with lysis and destruction, is similar to the action on staphylococci described by Rantz and Kirby (1944).

In Vivo

A series of animal experiments was planned to test: (1) whether infection of guinea-pigs by virulent leptospira could be prevented by the simultaneous administration of penicillin; (2) whether the course of the established disease could be affected by giving the drug once symptoms (fever or jaundice) had become apparent. Before these experiments were carried out Heilman and Herrell (1944) reported that large doses of penicillin, administered at frequent intervals beginning shortly after infection, will protect guinea-pigs against virulent leptospira. The aim of this work differed from our own, so preliminary experiments were undertaken on the lines originally intended.

It was found, however, that guinea-pigs inoculated intraperitoneally with virulent *L. icterohaemorrhagiae*, and given 60 units of calcium penicillin (also intraperitoneally) immediately thereafter and again 48 hours later, died in about the same time as untreated controls. This dosage corresponds to 14,000 units for a man of 70 kg. It was also found that penicillin had no therapeutic effect when treatment was begun after the onset of symptoms—i.e., on the sixth or seventh day after infection. In these experiments guinea-pigs received, in some cases, doses corresponding to 140,000 units for a man of 70 kg. In further experiments the administration of penicillin was started two to four days before symptoms were expected to appear. The results were irregular, and no definite conclusions could be drawn from them.

We finally carried out one experiment in an attempt to confirm the findings of Heilman and Herrell. Fifteen guinea-pigs were inoculated intraperitoneally with a virulent strain of *L. icterohaemorrhagiae*, and treatment with sodium penicillin suspended in an inert oil (arachis oil) was begun 18 hours later in 7 of the animals. Each received 200 o.u. at 10 a.m. and 5 p.m. and 400 units at 10 p.m. The dose was divided $\frac{4}{5}$ was injected subcutaneously and $\frac{1}{5}$ intraperitoneally. This treatment was continued for 13 days, and on the two following days 400 units were given at 10 a.m. and 5 p.m. Each guinea-pig thus received 12,000 units, corresponding to more than 1,000,000 units for a man of 70 kg.

The result was that 4 of 8 control guinea-pigs and 6 of the 7 treated animals survived. The seventh treated animal showed no signs of leptospiral infection at necropsy, and its tissues were not infective when inoculated into other guinea-pigs. So far as it goes, therefore, this experiment supports the view that penicillin, if given early enough, will reduce the death rate of infected guinea-pigs. In treatment of human beings the use of continuous therapy is a factor which could be expected to increase greatly the efficiency of the treatment and to allow it to be effective in later stages of the infection, by maintaining a steady concentration of the drug in the blood stream. In other ways the experience in guinea-pigs is valuable because human beings and guinea-pigs are very similar to one another in susceptibility to infection by virulent strains of *L. icterohaemorrhagiae* as well as in morbid anatomy of the disease produced.

Serological Tests

It seemed of interest to test the agglutinating titre of the serum, and the resistance to reinfection of those guinea-pigs which had recovered after penicillin treatment, compared with

the control animals which had withstood infection. It might be that the treated animals would show less immunity if the drug had reduced their contact with leptospiral antigen. No such tendency was apparent. The sera of 13 animals which had recovered in various experiments with penicillin treatment agglutinated *L. icterohaemorrhagiae* in dilutions of 1 in 30 or 1 in 100. Of 7 control animals, the sera of six gave similar titres while the seventh serum did not agglutinate at all. These agglutination levels are very low as compared with those of convalescent human cases, but we have found previously that guinea-pigs do not produce high titres after either infection or inoculation with leptospiral vaccines. Ten of the animals treated with penicillin were again inoculated with virulent leptospira. None of them showed any sign of infection, while all of four fresh control guinea-pigs died with typical symptoms.

These tests show that penicillin does not prevent the normal development of immunity in this infection.

Toxicity of Penicillin to Guinea-pigs

Heilman and Herrell (1944) found, in confirmation of Hamre *et al.* (1943), that penicillin in large doses is toxic to normal guinea-pigs. The former writers report that "young guinea-pigs receiving from 1,000 to 5,000 units of penicillin daily in divided doses frequently died after several days." Sodium and calcium penicillins from four different sources all produced this effect. Death was preceded by apathy, anorexia, loss of weight, and other signs, but the only obviously abnormal post-mortem finding was dilatation of capillaries throughout the tissues.

Heilman and Herrell believed that some of the infected guinea-pigs which died without the usual signs of leptospirosis were killed by penicillin. This may be so, but in our experience, especially when the course of the disease is unusually rapid, guinea-pigs sometimes die of leptospiral infection without showing either jaundice or haemorrhages, and the infection can be transmitted to other animals.

Rake *et al.* (1944) reached the conclusion that the rapid death of guinea-pigs after large doses of calcium penicillin was attributable to calcium. Solutions of calcium chloride in equivalent amounts were even more toxic. They also found that the toxic effect of sodium penicillin was greater in guinea-pigs than in rabbits or mice. This, however, was observed only after multiple injections, and death was delayed up to six days. The position is not yet clear, because Heilman and Herrell used calcium penicillin, and the toxic effects which they attribute to that drug were of the same kind as those which Rake *et al.* observed with the sodium salt.

The toxicity of the penicillin salt used in the last of our experiments was controlled as follows. Six normal guinea-pigs also received 800 units of sodium penicillin daily for 15 days. No change in weight, appetite, or behaviour was seen. Three weeks later the same animals were given 1,100 units of sodium penicillin twice daily for three and a half days. Again no ill effects were observed. This total dose of almost 20,000 units per animal would correspond to about 5 million units for a man of 70 kg.

Summary

Nine strains of *Leptospira icterohaemorrhagiae* (the causative organism of Weil's disease) and one of *L. canicola* (the cause of another form of leptospiral infection of men and dogs) were found sensitive to penicillin, which has a lethal as well as an inhibitory effect on multiplication.

Penicillin has a curative action on leptospiral infections in guinea-pigs, provided administration is begun very soon after infection.

The curative action of penicillin does not prevent the development, in guinea-pigs, of serum antibodies or resistance to reinfection.

Toxic action of penicillin in guinea-pigs, which has been reported by others, was looked for but was not found.

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