Steroid Diabetes in the Cat

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The background of these experiments may be recalled as follows. In 1936, Long and Lukens[1] described the amelioration of pancreatic diabetes by adrenalectomy. In 1938, Lukens and Dohan[2] increased the glycosuria of such doubly operated animals by large doses of adrenal cortical extract. In 1941, Ingle[3] described temporary diabetes in rats treated with corticotrophin or cortisone. In 1954, Abelove and Paschkis[4] compared the diabetogenic action of growth hormone and cortisone in different species and reviewed the bibliography, and Ingle[5] has reviewed the subject of steroid diabetes. The essence of these reports is that cortisone causes diabetes in rats[3] but is practically ineffective in dogs and cats.[4] When 9α-fluorohydrocortisone became available we tried it in nine intact cats and found in these preliminary experiments that this more potent synthetic steroid had a diabetogenic action in this species.

METHODS

9α-fluorohydrocortisone was kindly supplied by Merck and Co. and E. R. Squibb & Sons as a microcrystalline suspension. The usual dose of 5 or 10 mg. daily given subcutaneously was an arbitrarily chosen large quantity. Such doses are enormous amounts when compared to the 0.2 mg. which supports a human subject with adrenal insufficiency. When glycosuria had been well established for three to four weeks, it could be maintained on a lower dose of steroid. The animals were kept in metabolism cages and the daily urine volume was collected. The first appearance of glycosuria was determined by the glucose oxidase test paper (Tes-Tape-Lilly or Clinistix-Ames), and of urinary ketone bodies by the nitroprusside test. When glycosuria was present, the amount excreted per day was determined by Benedict's[6] method. Blood glucose was determined by Somogyi's[7] method on 0.1 ml. of blood obtained from the ear. The cats were fed weighed amounts of fresh (frozen) horse meat daily. Uneaten food was removed and weighed. Biopsies and intravenous glucose tolerance tests were performed under pentobarbital anesthesia. For the glucose tolerance tests, 0.5 gm. of glucose per kg. was given intravenously and blood sugars were taken at the times recorded.

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RESULTS

Table 1 shows that of nine cats treated with 5 or 10 mg. of fluorohydrocortisone daily, seven developed glycosuria, after five to twenty-seven days of treatment. The occasional delay in the appearance of glycosuria was also noted when some of the later courses of fluorohydrocortisone were given to cats 38 and 63. Of the cats listed in table 1, three died of diarrhea and pneumonia. In the others, the glycosuria ceased one to five days after the termination of treatment and they were used for other experiments except for two which were given prolonged, repeated courses.

Certain general effects of these large doses of fluorohydrocortisone should be mentioned. Within a day or two the appetite increased greatly. Two animals (38 and 63) developed a diuresis as reported by Swingle et al.[8] in dogs given 3 mg. per kg. which is comparable to our 10 mg. dose in 3 kg. cats. None of our animals developed gross edema.

After two to three weeks of treatment with either dose the abdomen became enlarged and at operation or autopsy contained enormous fat deposits. The limbs became thin and the animals slightly weak, although they behaved like well animals. The skin became so thin

TABLE 1

Effect of 9α-fluorohydrocortisone (FlF) in normal cats

Cat no.	FlF		Glycosuria		Blood sugar mg./100 ml.
	mg. per day	period days	appeared	on day	
6	5	31	No	—	—
6	10	11	No	—	—
7	10	35	Yes	5	159-180
14	5-10	43	Yes	27	180-210
37	5-10	57	Yes	7	128-190
43	5	8	No	—	—
51	5-10	18	Yes	6	105-147
64	5	10	Yes	6	—
38	5	89*	Yes	5	—
63	5-10	40*	Yes	14	170-191

*First course of treatment only—see text.

that one could not feel its resistance when giving a hypodermic. In four animals we confirmed the occurrence of atrophy of the adrenals described by others after this steroid. Like others[5] we have noticed an increased susceptibility to intercurrent infections in animals so treated.

Along with these general manifestations resembling Cushing's syndrome, glycosuria developed, with 1 to 2 gm. of glucose excreted daily. The fasting blood sugar was elevated little or none. The delay of one to two weeks in the development of hyperglycemia after the appearance of sugar in the urine is attributed to the slight effect of corticoids on the renal threshold for glucose.[9] Gradually the glycosuria increased in amount and the blood sugar levels rose to 200 mg. per 100 100 ml. or more.

The two animals treated with long and repeated courses of steroid will be presented in more detail. During the first treatment period of eighty-nine days, Cat 38 ate ravenously. When this course of steroid was terminated, the appetite fell remarkably. A week after the cessation of steroid, Cat 38 went for five days without eating at all, although it was lively and well. This behavior followed the termination of later courses of steroid.

Figure 1a is from a pancreatic biopsy of Cat 38 taken at the end of the first eighty-nine day course of steroid. It shows that at the end of almost three months of glycosuria and hyperglycemia (140-272 mg./100 ml.) the animal had developed severe hydropic degeneration of the β-cells. Six weeks later, when the cessation of steroid and the reduced food intake had led to the disappearance of glycosuria, the islands appeared normal (figure 1b). The development of hydropic degeneration and the subsequent anatomical and functional recovery after the removal of the diabetogenic stress, follow the course of events previously described in this species. In the past, the hyperglycemia caused by partial pancreatectomy,[10] growth hormone,[11] and massive doses of glucose[12] have been associated with the production of similar lesions and with their reversibility when treatment with insulin, diet or phlorhizin was begun early enough. In the present experiments, the production of glycosuria and hyperglycemia by a steroid was also accompanied by β-cell degeneration.

In addition to the first course of treatment and first rest period described above, two cats received numerous irregular courses of steroids for the purpose of testing the diabetogenic activity of other steroids and to observe the results of repeated bouts of this form of temporary diabetes. Table 2 summarizes the events in these animals.

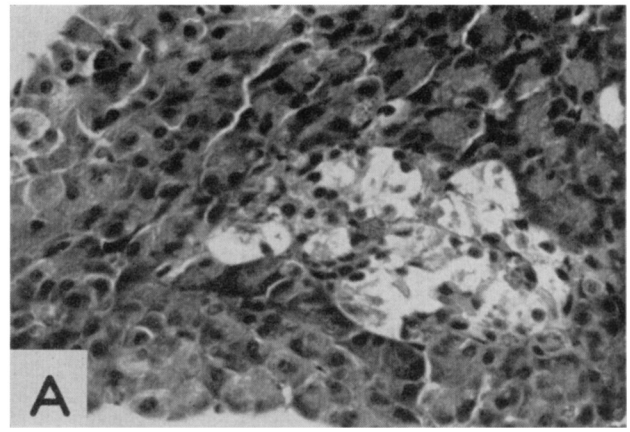
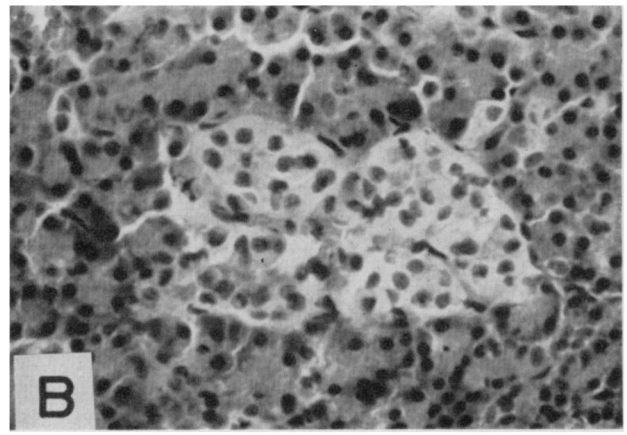


FIG. 1. A. Cat 38. Island of Langerhans showing marked hydropic degeneration at the end of the first course of treatment with fluorohydrocortisone (x 290).
B. Islet of same animal six weeks after the cessation of steroid treatment. The recovery from diabetes was accompanied by the return of the islands to normal appearance (x 290).



It shows that in Cat 38 there were 253 days of steroid administration and 335 days of rest periods in the course of almost twenty months. Cortisone and hydrocortisone were unable, but prednisone was able to cause glycosuria in the doses employed. In the last treatment period, a small dose (1.2 mg. daily) of fluorohydrocortisone was used as a form of tolerance test. This amount has not caused glycosuria in normal animals. The fact that it caused glycosuria at the end of these repeated bouts of steroid treatment led us to assume that some permanent reduction of the pancreatic reserve had taken place during the preceding treatment. The courses of treatment with fluorohydrocortisone in Cat 63 were likewise followed by susceptibility to the small test dose of 1.2 mg. daily of fluorohydrocortisone.

In general, the weight of these animals was constant during the periods of steroid treatment, increased slightly after stopping the drugs and then stabilized.

TABLE 2

Animals given repeated courses of steroids

Treatment period days	Steroid	Average mg./day	Glycosuria	Rest period days	Glycosuria disappeared days
	Cat 38				
89	Fluorohydrocortisone	4.2	Yes	56	15
18	"	2.5	Yes	31	3
8	Hydrocortisone	5.0	No	1	—
9	Fluorohydrocortisone	2.5	Yes	20	2
9	Cortisone	50.0	No	30	—
22	Prednisone	50.0	Yes	56	27
51	Fluorohydrocortisone	4.0	Yes	16	13
37	"	4.0	Yes	115	34
10	"	1.2	Yes	10	8
253	← Total period days →			335	
	Cat 63				
40	Fluorohydrocortisone	8.5	Yes	46	5
87	"	8.0	Yes	29	29
22	"	2.5	Yes	118	40
12	"	1.2	Yes	8	2
161	← Total period days →			201	

Body weight was fairly constant during treatment with fluorohydrocortisone, when food intake was large. Fat depots became enormous and muscle wasting occurred. This situation suggests that this steroid causes changes in the utilization of the diet so that protein is wasted and fat is spared. In addition, there were times when the glycosuria reached amounts which would cause slight loss of weight.

During the next to last rest periods of Cats 38 and 63 which are shown in table 2 (115 and 118 days respectively), but after the disappearance of the last post-treatment period of glycosuria (thirty-four and forty days respectively), the glucose tolerance tests which are summarized in table 3 were performed. The slight delay in the return to normal of the blood sugar level in these animals subjected to long-term treatment with fluorohydrocortisone is regarded as borderline, or suggestive evidence of the incomplete recovery of these animals.

Cats 38 and 63 were sacrificed (under Nembutal) after the last rest periods recorded respectively in table 2. At this time they were in excellent condition. The findings in both animals were so similar that a common description will suffice. Grossly, there were enormous abdominal, omental, and perirenal fat depots but average amounts of subcutaneous fat. The pancreas was grossly normal except for the well-healed scar of previous biopsies at its tail. Pancreatic weights of 10 and 7 gm. were in the normal range. In Cat 38, after its first twenty-seven days on fluorohydrocortisone, one adrenal was removed at the first biopsy. This gland weighed 129 mg. and showed slight atrophy of the inner zones, At autopsy, the remaining adrenal had not only failed to undergo the usual hyperplasia, but weighed only 94 mg. In contrast, the average weight of both adrenals in fifteen normal cats was 586 mg.[12] In Cat 63, both adrenals weighed 149 mg., again illustrating the marked atrophy produced by this steroid. In both animals the bone of the skull was thin and soft, indicating gross osteoporosis. At biopsy during steroid treatment, even more than at autopsy, the skin was about one-fourth the thickness of a comparable normal area.

On microscopic section, the *adrenals* showed marked atrophy of the inner zones. In both cats there was a collagenous zone about the medulla which suggested that the type or severity of the atrophy from fluorohydrocortisone differed from that seen after hypophysectomy or shorter courses of steroid. In the *pancreas* the acinar tissue, ducts, and vessels were normal. The islands were reduced in number and size on survey of numerous sections. Except for occasional hydropic cells, the islets appeared normal in that the α and β cells were present in them. Some irregularity of shape, especially of the small islands, was noted. The paucity of islands and their smaller average size on examination of many fields is regarded as early or mild atrophy of the islands.

The kidneys appeared normal, a finding which contrasts with the changes described in rabbits treated with

TABLE 3

Glucose tolerance tests on two steroid-treated cats (0.5 gm. glucose per kg. intravenously)

Cat No.	Blood glucose at hours: 0	½	1	2	3	4
	mg. per 100 ml.					
63 (N=3)	84	241	216	184	140	110
38 (N=2)	130	223	201	172	149	127
Normal cats (N=7)						
Mean	95	232	195	147	107	88
S.E.M.	±5	±10	±12	±14	±9	±6

N = Number of tests.

cortisone.[16] The heart, coronary vessels, liver, pituitary and thyroid all appeared normal. The skin, especially at earlier biopsies, showed atrophy of the dermis and of the inner layers of the epidermis.

DISCUSSION

Incidence of glycosuria. One animal required twenty-seven days of steroid treatment before the first appearance of glycosuria and only one of the two animals, which failed to develop glycosuria, was treated for this long a time. Under such circumstances, the true incidence of glycosuria and the variety of individual response in normal cats require further study. In two dogs[8] comparable large doses of fluorohydrocortisone (3 mg. per kg. per day) failed to cause hyperglycemia in three to fourteen days. In view of our experience this trial may be too brief to be conclusively negative for a diabetogenic effect in the dog.

The development of hydropic degeneration of the β-cells in response to the administration of a steroid hitherto untested in this way may be viewed in relation to the following observations. (a) It seems probable that the mechanism by which hyperglycemia is produced by fluorohydrocortisone is the same as that exerted by cortisone except in the degree or intensity of the effect. (b) If this be so, much if not all of the initial metabolic disturbance is due to extrapancreatic effects of the steroid. This has been shown directly by the intense diabetogenic action of cortisone in hypophysectomized-depancreatized animals. (c) As recently outlined by Ingle,[5] the extrapancreatic effects include some increase in gluconeogenesis and strong indirect evidence for some inhibition of the utilization of glucose. (d) If the extrapancreatic effects leading to glycosuria and hyperglycemia are the same in the rat and cat, one may note the striking differences between these two species in the response of the islands of Langerhans to a common stress. Ingle[5] has described the temporary diabetes, the evidence of hypertrophy of the islands and the virtual absence of hydropic degeneration in the rat. In cats, we have observed the development of hydropic degeneration of the islands after an appropriate period of diabetes. The recognition of such variations in the behavior of the islands of different species is not new, but each new demonstration of this ought to make one more aware of the possible range of behavior of the islands in man. One report from this laboratory[14] suggests that a variety of appearance and functional capacity of the islands may occur in man.

To date, hydropic degeneration of the islands in the cat has accompanied four procedures, namely, subtotal pancreatectomy,[10] pituitary or growth hormone diabetes,[11] diabetes produced by the administration of glucose,[12] and steroid diabetes. One may assume (a) that in these experiments four separate damaging agents or procedures have been discovered, or (b) that these forms of diabetes with the same lesion are the result of some factor (or factors) which is common to all four procedures. Either hypothesis will be best applied in designing further observations.

In our opinion both animals which had prolonged steroid treatment were left with some impairment of the normal capacity to handle carbohydrate three to four months after the cessation of large doses of steroid. The slight deviation of the sugar tolerance tests, the development of glycosuria on 1.2 mg. per day of fluorohydrocortisone, and the probable atrophy of the islands of Langerhans lead to this suggestion.

The abnormalities resembling those of Cushing's syndrome are obviously not the rule in human diabetes. However, the need for prolonged steroid treatment, the slow development of hyperglycemia, the remarkable influence of fluorohydrocortisone on appetite, the obesity, and finally the mild form of the diabetes may be of interest to students of the disease in man.

SUMMARY

By means of large doses of fluorohydrocortisone, glycosuria and hyperglycemia have been produced in seven of nine cats, a species resistant to those steroids hitherto tested. The islands of Langerhans responded with hydropic degeneration as in other forms of experimental diabetes in cats. In the only two animals in which prolonged treatment was given, the island lesions progressed to early atrophy and residual impairment of carbohydrate metabolism was demonstrable. The physiological implications of a new means of producing islet injury are briefly discussed.

SUMMARIO IN INTERLINGUA

Diabete Steroide in Cattos

Per medio de grande doses de fluorohydrocortisona, glycosuria e hyperglycemia esseva producite in septe ex nove cattos, ben que iste specie esseva resistente al steroides previemente essayate. Le insulas de Langerhans respondeva per degeneration hydropic, como illos lo face in altere formas de diabete experimental in cattos. Tractamento prolongate esseva applicate a solmente duo del animales. In illos, le lesiones del insulas progredeva a un atrophia rapide, defectos residue del metabolismo de hydrato de carbon esseva demonstrabile. Es presentate un breve discussion del signification physiologic de iste discoperta de un nove methodo de effectuar lesiones del insulas.

DISCUSSION

ARTHUR R. COLWELL, SR., M.D., (*Chicago*): This is a most interesting experimental demonstration. The biopsy findings are very convincing, and temporary diabetes seems to have existed beyond question. I cannot help but wonder, however, about the conclusion that a mild permanent diabetes was produced. This is probably a matter of definition, but steroids were still used late in the period of study, some weeks after the discontinuance of the heavy dosage, and differences in the glucose tolerance tests were not striking.

How much glycosuria remained after discontinuance of the large dosage of steroid compared with the amount present during the period of heavy dosage?

ARNOLD LAZAROW, M.D., PH.D., (*Minneapolis*): How can you explain the differences in the diabetogenic potency of fluorohydrocortisone and cortisone? Is this due to the fact that you can administer larger doses of the fluorohydrocortisone without producing undesirable side effects?

JOHN BUSE, M.D., (*Philadelphia*): I agree with Dr. Colwell that it is a matter of definition whether these animals can be called permanently diabetic. Various months after the suspension of the therapeutic doses of fluorohydrocortisone the cats were aglucosuric and had normal fasting blood sugars. However, in addition to the evident histological lesions of the pancreas, a mild functional impairment was demonstrated by the rapid appearance of glycosuria and hyperglycemia with one-tenth of the dose of fluorohydrocortisone that was necessary to provoke the same effects in normal cats.

In reference to Dr. Lazarow's question we have treated cats with subcutaneous injections of cortisone, hydrocortisone and delta-1-cortisone, using large doses. We have not quantitated the difference in diabetogenic potency of these various steroids, but there is no doubt that milligram for milligram fluorohydrocortisone was much more potent than any of the aforementioned steroids.

REFERENCES

1 Long, C. N. H., and Lukens, F. D. W.: The effects of adrenalectomy and hypophysectomy upon experimental diabetes in the cat. J. Exper. Med. *63*:465-90, 1936.

2 Lukens, F. D. W., and Dohan, F. C.: Further observations on the relation of the adrenal cortex to experimental diabetes. Endocrinology *22*:51-58, 1938.

3 Ingle, D. J.: The production of glycosuria in the normal rat by means of 17-hydroxy-11-dehydrocorticosterone. Endocrinology *29*:649-52, 1941.

4 Abelove, W. A., and Paschkis, K. E.: Comparison of the diabetogenic action of cortisone and growth hormone in different species. Endocrinology *55*:637-54, 1954.

5 Ingle, D. J.: Experimental steroid diabetes. Diabetes *5*:187-93, 1956.

6 Benedict, S. R.: The detection and estimation of glucose in urine. J.A.M.A. *57*:1193-94, 1911.

7 Somogyi, M.: Determination of blood sugar. J. Biol. Chem. *160*:69-73, 1945.

8 Swingle, W. W., Baker, C., Eisler, M., Le Brie, S. J., and Brannick, L.: Maintenance of adrenalectomized dogs with 9-alpha-halo adrenal and other steroids. Endocrinology *57*:220-30, 1955.

9 Conn, J. W., Louis, L. H., and Wheeler, C. E.: Production of temporary diabetes mellitus in man with pituitary adrenocorticotropic hormone: relation to uric acid metabolism. J. Lab. and Clin. Med. *33*:651-61, 1948.

10 Homans, J.: Degeneration of the islands of Langerhans associated with experimental diabetes in the cat. J. Med. Res. *30*:49-69, 1914.

11 Cotes, P. M., Reid, E., and Young, F. G.: Diabetogenic action of pure anterior pituitary growth hormone. Nature *164*: 209-11, 1949.

12 Dohan, F. C., and Lukens, F. D. W.: Experimental diabetes produced by the administration of glucose. Endocrinology *42*:244-62, 1948.

13 Bloodworth, J. M. B., and Hamwi, G. J.: Experimental diabetic glomerulosclerosis. Diabetes *5*:37-42, 1956.

14 Lukens, F. D. W., and Dyer, W. W.: The relation between appearance and behavior of the islands of Langerhans in man. Am. J. Med. Sci. *231*:313-19, 1956.