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Resistance of pigeons to the lethal action of iletin (insulin) with observed effects on reproduction.

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The pancreatic extract used by us is prepared under the name iletin (insulin, Lilly); it was supplied by the makers and bore the date of preparation. It is prepared by the method of Banting[1] and Macleod[2], one unit of the extract being the amount necessary to reduce the blood sugar of a kilogram rabbit to 0.045 per cent. At this percentage the rabbit often goes into convulsions and death sometimes follows. This unit of iletin or insulin may therefore be considered the lethal dose for a kilogram rabbit.

We are using the pancreatic hormone as a part of a more general study on the relation of the various incretions to reproduction and to sex. Our specific purpose here was to learn whether as a consequence of the hypoglycemia induced by this extract all ovulations would be suppressed in the pigeon by a dosage which should leave the other conditions necessary for reproduction essentially undisturbed. I have earlier reported[3] a marked hypertrophy of the suprarenals at the ovulation period, and in collaboration with Honeywell[4] have shown that parallel with this hypertrophy there regularly occurs in pigeons a marked increase of blood sugar coincident with ovulation. Other unpublished work by Honeywell and myself has made it clear that in each of two very common causes of suppressed ovulation the blood sugar is abnormally low. Such earlier observations have led to the view that a low blood sugar tends to suppress ovulation in birds. This conception is again supported by the results of the present study. It is found that

1 Banting and Best, *Jour. Lab. and Clin. Med.*, 1922, vii, 464.

2 Banting, Best, Collip, Macleod and Noble, *Amer. Jour. Physiol.*, 1922, lxii, 162.

3 Riddle, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 122.

4 Honeywell and Riddle, PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 280.

most ovulations are successfully suppressed by subcutaneous injections of quantities of insulin which certainly leave the birds feeding and mating normally, and which do not affect the body weight more adversely (average initial weight = 176g.) than do some other tested tissue extracts which do not suppress ovulation. This depression (12g.) is also not more than the maximum normal seasonal variation.

TABLE I.

Ovulations suppressed in ring doves during one month by subcutaneous injections with one-sixth unit iletin twice daily.

Bird No.	Loss in weight (grams).	Number of ovulations.	
		Expected.	Realized.
1	11	6	0
2	13	6	0
3	4	6	2
4	16	4	0
5	14	4	2
6	14	4	0
7	13	4	0
8	14	4	0
9	15	2	0
10	7	2	0
Total or Average..	12	42	4

Table 1 indicates that not more than one-tenth of the expected number of ovulations are realized under the dosage selected. The indicated number of suppressed ovulations is, however, somewhat too high since only one of the 10 birds used was given blank injections during the control period (upon which the ovulation rate is calculated). Much earlier experience with blank injections and with injections of other tissue extracts shows that this treatment alone appreciably reduces the ovulation rate. It seems certain, however, that the ovulations actually suppressed were equal to more than one-half the number indicated in the table. Only very tame doves were used in this study.

In addition to the data of the table it has been found that this dosage usually does not prevent the ovulation of an egg which is within 48 hours of ovulation at the time of beginning the injections. Again, five birds killed or opened for inspection at three to twenty days after beginning injection showed three cases of degenerating (larger) ova, and two cases in which no ovum has been able to pass into the final stage of

rapid growth.[5] It is thus clear that this quantity of insulin blocks ovulation both by preventing ova from beginning their final period of rapid growth and by sometimes causing them to be resorbed after having entered this stage of growth.

The data (sugar curve for 10 birds) of the accompanying paper[6] show that in the suppressed ovulations of our table 1 the blood sugar was lowered to about .080 per cent.—or one-half its normal value—for a period not longer than four hours twice daily. Depression of the blood sugar to this extent is sufficient to suppress many or most ovulations in ring doves.

In making the necessary tests for the proper dosage for the above purpose it was further learned that the pigeon shows a marked resistance to the lethal action of the extract. This fact

TABLE II.

The resistance of doves and pigeons to large subcutaneous doses of insulin. Birds not permitted to eat until 48 hours after injection. (Upper part of table = ring doves; lower = common pigeons).

No. of bird.	Amount of iletin.		Time (hours) no food before injection	Death or survival and remarks.
	Units.	X lethal for rabbit.		
1	5.0	33	21	Died after 5 days; took no food.
2	4.5	30	22	Survived.
3	4.8	30	7	Survived.
4	4.7	30	7	Died* after 28-34 hours.
5	5.0	30	22	Survived.
6	5.0	30	22	Survived.
7	3.2	20	5	Survived.
8	1.3	10	21	Died* after 36-42 hours.
9	1.7	10	0	Survived.
10	1.8	10	0	Survived.
11	1.5	10	0	Survived.
12	1.5	10	0	Survived.
13	1.0	6	0	Survived.
14	1.0	6	0	Survived.
15	1.0	6	0	Survived.
16	10.5	33	0	Died after 8.5 hours.*
17	5.8	18	22	Survived.
18	5.7	18	20	Survived.
19	3.5	10	22	Survived.
20	3.4	10	20	Survived.

*These birds had needle-puncture of heart, or were bled from beak, to obtain sugar samples after injection; this bleeding possibly partly responsible for death.

[5] Riddle, *Amer. Jour. Physiol.*, 1916, xli, 387.

is of interest in connection with studies elsewhere now being made upon insulin, since it seems probable that some effects of the hormone scarcely observable in mammals may be successfully studied in the pigeon. Table 2 records our chief observations on this point except for data concerning symptoms and extent of the hypoglycemia induced by heavy dosage.

Certainly some of the symptoms observed in rabbits[2] are sometimes duplicated in the impaired vision and incoördinate movements of the heavily dosed pigeon. These movements strongly resemble those of the ataxic pigeon. Convulsions were observed only in three of the four birds killed by the extract. Unlike the rabbit the bird becomes quiet, apparently very tame (really impaired vision?), and gives little or no evidence of hunger for many hours or days. Though the intestines of most of our heavily dosed birds were made free of food before injection, and though none were permitted to take food earlier than 48 hours after dosage, some birds had to be forcibly fed (impaired vision?) even at the end of 72 or 96 hours. No abscesses have resulted from more than 700 injections.

Two lots or shipments of the extract have been used. The data of table 2 were obtained almost equally from the use of the two preparations. The data of Table 1 are all from the first lot kept in a refrigerator and used when aged one to two months. Its capacity to lower the blood sugar at the end of two months, in one-sixth unit doses, was somewhat less than that of the second lot as tested against the latter within 48 hours after its arrival at the laboratory; the values obtained being .082 per cent. sugar for lot 1, and .062 per cent. sugar for lot 2. These data were obtained as part of the studies reported in the accompanying communication.[6] Other data obtained in connection with that study indicate that though the normal blood sugar of all the kinds of pigeons used is notably higher than that of the rabbit, the value in the pigeon was sometimes reduced to .020 to .040 per cent. without convulsions or death. Bird No. 16 which died after 8.5 hours, gave single unchecked values of .020 per cent. two hours after dosage and .010 per cent. after four hours. Another of the birds which died 28-34 hours after injection gave a value of .025 per cent. after twenty-one hours. Two birds survived though they gave sugar values of .020 and of .030 and .040 per cent.

[6] Honeywell and Riddle, PROC. SOC. EXP. BIOL. AND MED., 1923, xx, No. 5.