EFFECT OF BENACTYZINE AND GANGLERON ON EMBRYOGENESIS OF HENS

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Injection of benactyzine in doses of 0.01-1 mg into 8-day chick embryos causes higher mortality than doses of 10 and 20 mg on the 2nd-10th days after injection. Gangleron, in doses of 10 and 20 mg per egg, often causes malformations of the same type.

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Central cholinolytics have found a wide application in clinical medicine, but the study of their teratologic action is essentially only just beginning.

The object of the investigation described below was to study the effects of the muscarinolytic drug benactyzine and of the nicotinolytic drug gangleron* on the development of chick embryos, which are convenient objects for teratologic research.

EXPERIMENTAL METHOD

The drugs, in doses of from 0.01 to 20.0 mg per egg, dissolved in 0.1 ml physiological saline, were injected into the yolks of 8-day chick embryos. The control group received 0.1 ml physiological saline. The eggs were incubated for up to 22 days in a thermostatically controlled room at 37° and at a relative humidity of 60%. As a result of daily ovoscopy the dead embryos could be removed and the time of death established; on the first day (from the injection and the local irritant properties of the drug) and on the 2nd-10th day after injection. Anomalies of the dying embryos were revealed by external inspection. The number of chickens which hatched out also was recorded.

The percentage of embryos dying on the first day after injection of the drugs was calculated relative to the number of eggs in the group, and all other indices relative to the number of embryos not dying on the first day after injection. All data were analyzed by statistical methods, with determination of the means and of their 95% confidence limits, with the aid of Genes's tables [1].

EXPERIMENTAL RESULTS

Experiments were carried out on 980 embryos. The results are given in Table 1.

No difference was found between the number of embryos dying on the first day after injection of benactyzine in all the doses tested, of gangleron in doses of 0.01, 0.1, and 1 mg per egg, and of physiological saline. After injection of gangleron in doses of 10 and 20 mg per egg, however, the mortality on the first day was much higher, presumably because of its strong local irritant action [2].

On the 2nd-10th day after injection in a dose of 0.01 mg per egg, benactyzine caused death of 87% of the embryos (compared with 28.6% after injection of physiological saline), but if a larger dose was given the number of embryos dying in this period was reduced (17% after injection of 10 mg per egg). With a further increase in the dose the mortality among the embryos in this period rose slightly. Gangleron, in a dose of 0.01 mg per egg, gave the same percentage mortality among the embryos on the 2nd-10th day

*1,2-Dimethyl-3-diethylaminopropyl-p-isobutoxybenzoate hydrochloride.

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TABLE 1. Effect of Gangleron and Benactyzine, Injected in Different Doses into 8-Day Chick Embryos, on Embryogenesis (mean data and 95% confidence limits)

Drug	Dose (in mg per egg)	No. of eggs	Percentage of embryos dying after injection of drug		Hatching rate (in %)	External anomalies (in %)	
			on 1st day	on 2nd-10th day		early form of micromelia	other malformations
Benactyzine	0,01	70	0 0—5	87 77—94	3 0—10	27 17—39	13 6—23
	0,1	68	0 0—5	75 63—85	3 0—10	26 16—39	20 12—32
	1,0	95	0 0—4	52 42—63	7 .3—14	32 23—43	10 5—18
	10,0	99	6 2—13	17 10—26	10 4—17	9 4—16	1 0—6
	20,0	45	7 1—18	19 9—34	7 2—20	10 3—23	2 0—13
Gangleron	0,01	68	0 0—5	32 22—45	7 2—16	12 5—22	6 2—14
	0,1	70	3 0—10	13 6—24	15 7—25	2 0—8	3 0—10
	1,0'	93	3 1—9	22 14—32	4 1—11	17 10—26	6 2—12
	10,0	100	40 30—50	30 18—43	5 1—14	18 10—30	48 35—61
	20,0	45	80 65—90	56 21—86	0 0—34	44 14—79	33 8—70
Physiological saline		227	6,1 3,0—9,2	28,6 22,5—34,7	30,5 24,3—36,7	13,0 8,5—17,5	1,4 0—6

after injection as physiological saline; with a dose of gangleron of 0.1 mg per egg, this index showed a significant decrease. With a further increase in the dose of gangleron the number of dying embryos again increased.

The fact that a smaller dose caused the death of a larger number of embryos is interesting. A similar phenomenon has been described for D-amphetamine in experiments on male albino mice [3]. It is impossible to give any satisfactory interpretation of this phenomenon at the present time.

The hatching rate was lower after injection both of benactyzine and of gangleron than in the control, but it showed a tendency to rise after injection of benactyzine in a dose of 10 mg per egg and gangleron in a dose of 0.1 mg per egg (the same doses as those after injection of which fewest embryos died on the 2nd-10th days). Gangleron, in a dose of 20 mg/egg, caused a tendency for the hatching rate to decrease.

An early form of micromelia of the embryos was observed significantly more often than in the control after injection of small doses of benactyzine (0.01-1 mg per egg), In doses of 10 and 20 mg per egg it had the same action as physiological saline. Gangleron, in a dose of 0.1 mg per egg, caused this anomaly much less frequently. In doses of 0.01, 1.0, and 10 mg per egg its effect was indistinguishable from that of the control, while in a dose of 20 mg per egg, an early form of micromelia was seen more often.

Under the influence of small doses of benactyzine (0.01-1 mg per egg) malformations developed significantly more often than in the control, and they were varied in character (various anomalies of the beak, cataract), but injection of benactyzine in doses of 10 and 20 mg per egg have results indistinguishable from the control. Gangleron, in small doses (0.01-1 mg per egg), did not cause an increased incidence of malformations, but doses of 10 and 20 mg per egg led to a sharp increase in their frequency, the same anomalies being observed in all cases: "parrot" beak; marked micromelia; deformities of the lower limbs (Fig. 1). Most of the embryos died on the 20th day of incubation, but some were still alive on the 22nd day.

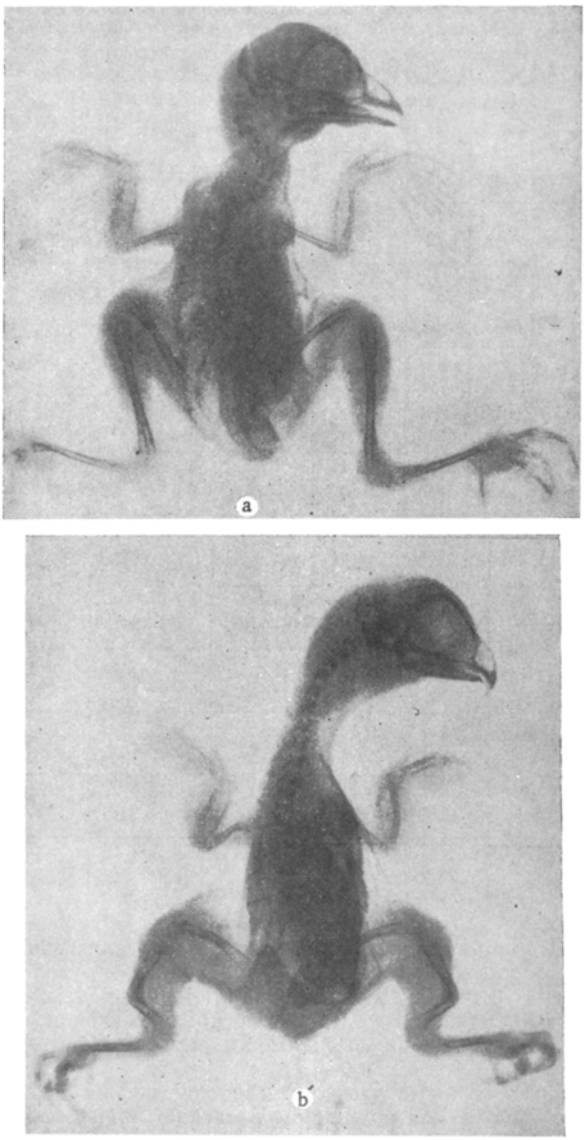

Fig. 1. Roentgenogram of a 21-day chick embryo: a) normal; b) typical malformation caused by injection of gangleron in a dose of 10 mg per egg.

Small doses of benactyzine thus proved more toxic than large; the embryos which died exhibited an early form of micromelia and various other malformations. Since the malformations were not constantly of the same type, there are no grounds for considering that benactyzine is a teratogenic compound.

The effect of gangleron, expressed as a decrease in the mortality rate to below the control level, during the period from the 2nd to the 10th day after its injection, was exhibited in a dose of 0.1 mg per egg. With an increase in the dose the toxic effects of the drug became stronger: a higher proportion of the embryos died, the hatching rate was reduced, the incidence of an early form of micromelia was increased; in doses of 10 and 20 mg per egg, the same type of malformation was observed very frequently and constantly. Because of this, the possible teratogenic action of gangleron must be studied widely in animals of different species.

LITERATURE CITED

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