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Effects of Scopolamine Hydrobromide on the Development of the Chick and Rabbit Embryo

W. G. McBride, P. H. Vardy and J. French

Foundation 41, Developmental Biology Unit, The Women's Hospital, Surry Hills, N.S.W. 2010.

published in *Aust. J. Biol. Sci.*, 1982, **35**, 173–8.

Note on the Paper 'Effects of Scopolamine Hydrobromide on the Development of the Chick and Rabbit Embryo' by W. G. McBride, P. H. Vardy and J. French

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The Journal wishes to bring the following to the attention of readers.

After allegations of fraud were made publicly about the articles cited above, published in the *Australian Journal of Biological Sciences*, a Committee of Inquiry was established by the Board of Foundation 41 to investigate the allegations. The Committee published a report on 2 November 1988 which concludes as follows:

'(a) Deliberate falsification did occur in the paper "Effects of Scopolamine Hydrobromide on the Development of the Chick and Rabbit Embryo" published in the Australian Journal of Biological Sciences, 1982, Vol. 35, pp. 173–8, in that it was falsely stated in that paper—

i. That eight rabbits were the subject of the experiment in New South Wales when only six rabbits were the subject of the experiment in that State.
ii. That the dosages shown under the heading "Oral Ingestion" in Table 2 were correct.
iii. That the foetuses in the experimental group were sectioned.
iv. That eight rabbits contemporaneously obtained from the same suppliers and fed the same diet were used as controls.

'(b) The experiment mentioned in that paper was not conducted in accordance with proper scientific method and was not honestly reported.

'(c) Although criticisms regarding inappropriate controls may be made, we have not determined that deliberate falsification occurred in or in connection with the note published in the Australian Journal of Biological Sciences, 1983, Vol. 36, pp. 171–2.

'(d) The above experiment and its published results does suggest that in relation to the publication of those results Dr. McBride was lacking in scientific integrity.'

The report states that, on 20 September 1982, Ms J. French recorded in a letter to Foundation 41 that her name had, without her knowledge, been used as co-author of the paper which contained statements and data with which she disagreed. On 18 October 1982, seeking employment, Mr P. H. Vardy circulated his C. V. to all members of the Australian Teratology Society, stating that the paper had been written and submitted without the knowledge of either Ms J. French or himself.

The report is signed by the three members of the Committee of Inquiry: the former Chief Justice of the High Court of Australia, Sir Harry Gibbs (chairman); Professor Robert Porter, director of the John Curtin School of Medical Research at the Australian National University; and Professor Roger Short of the Faculty of Medicine, Monash University.

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Abstract

When 100 or 200 μg of scopolamine hydrobromide were injected into hen eggs after they had been incubated for 96 h the major malformations of the embryo found were gastroschisis, exencephaly, reduction deformities of the limbs, microphthalmia and buphthalmia when the eggs were opened on the 12th day of incubation.

Eight foetuses of one of eight New Zealand white rabbit does given scopolamine hydrobromide at the rate of 473 μg/kg in their drinking water from the 10th to the 14th day of gestation showed malformations consisting of microphthalmia, buphthalmia, exencephaly and hydrocephaly. A second doe which similarly received 520 μg/kg of the hydrobromide had four malformed foetuses showing microphthalmia and had two foetuses which were resorbed. However, there were no malformations in foetuses of eight does given bolus doses of scopolamine hydrobromide ranging from 37 to 184 μg/kg by intramuscular injection every 12 h from the 10th to the 14th day of gestation.

Results indicate interference by the drug with the function of the cholinergic nerves on the development of the chick and rabbit embryo.

Introduction

Hamburger (1939), from his transplant experiments, concluded that morphogenesis is independent of innervation, and that atypical or completely abnormal morphogenetic development in a large percentage of transplants cannot be attributed to deficient nerve supply. However, recent research has indicated that neural function may be more important than previously acknowledged. Kieny and Fouvet (1974) found that excision of a portion of the spinal cord in 2-day chick embryos, although made rostral to the prospective leg level, produced a high frequency of pre-axial hemimelia and a lower proportion of post-axial hemimelia. They concluded that the question of the influence of the nervous system in limb development is open for further investigation. Excision of the part of the brachial section of the neural tube at stages 13–15 (Hamburger and Hamilton 1951) of development was found to produce deformity of the wing (Čihák *et al.* 1978). Similarly, ipsilateral leg deformities were produced by excision of portion of the lumbar neural tube at stages 13–16 (McBride 1979). Singer (1943) has shown the importance of nerve supply in the regeneration of the forelimb of the adult *Triturus*, although Yntema (1959) was able to obtain the growth of the limb in aneurogenic *Amblystoma* larvae.

Since operative interference with the nerve supply of the developing chick results in limb malformations it was decided to study the effects of pharmacological

interference with the function of muscarinic receptors of the cholinergic nerves in the chick and rabbit embryo. The chick embryo lacks a choriodecidual placenta; therefore, it is not the ideal model for studying the possible teratogenic effects of drugs on an embryo. The rabbit embryo was therefore also used in this study.

Materials and Methods

The eggs used were from a flock of White Leghorns (Inghams Poultry Farms, Casula, N.S.W.). A total of 300 eggs were incubated at $38 \pm 0 \cdot 1$°C and 70–80% R.H. in a Qualtex commercial incubator. After incubation for 96 h, two batches of 100 eggs were injected respectively with 100 and 200 μg of scopolamine hydrobromide (Boots Company Australia Pty Ltd) using a 1-ml tuberculin syringe. The drug, which had been prepared so that 1 litre of sterile isotonic saline contained 1 g, was injected into the yolk sac. In addition 100 eggs were injected with 400 μl of isotonic saline to act as controls. The injection sites were sealed with wax and the eggs were opened on the 12th day of incubation.

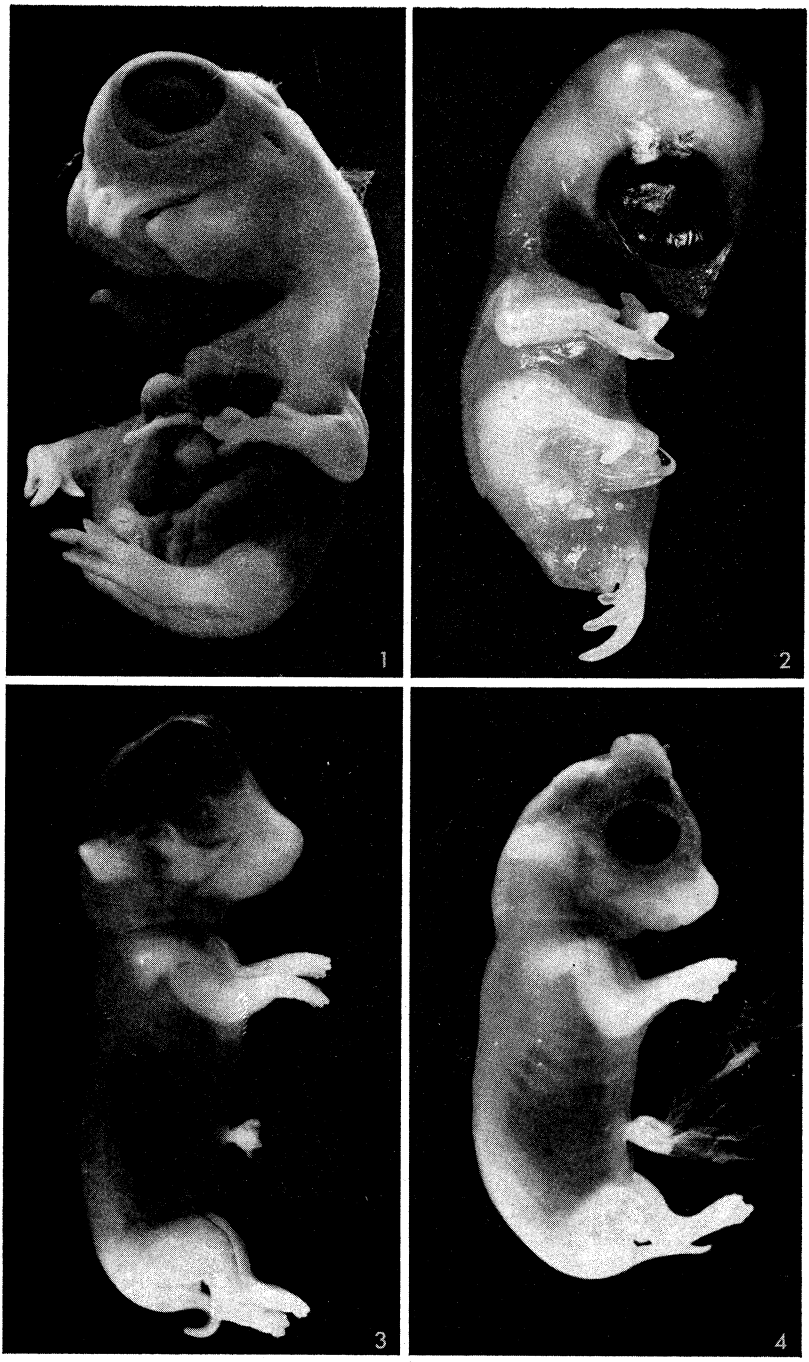
In the second part of the experiment, 24 virgin New Zealand white rabbit does (University of New South Wales animal colony), weighing between 2·62 and 4·03 kg were mated with healthy males of proven fertility. Copulation was observed and the day of mating was designated gestation day 1. The mated females were individually housed in stainless-steel cages and were provided with rabbit chow (Allied Mills Ltd, Rhodes, N.S.W.), green vegetables and water *ad libitum*. The water used throughout the experiment was from the Sydney City Water Supply which is known not to contain teratogens.

Eight does were given scopolamine hydrobromide by intramuscular injection at 12-hourly intervals (8 a.m. and 8 p.m.) from the 10th to the 14th day of gestation inclusive. One doe which received 137 μg/kg twelfth hourly died on the second day of medication, 10 min after injection; another receiving 185 μg/kg twelfth hourly died 30 min after the first injection. Bolus doses of scopolamine hydrobromide caused distress to the does, due to rapid increase in heart rate. The second batch of eight does was given scopolamine hydrobromide in the drinking water, from the 10th to 14th day of gestation inclusive. The amount of drinking water, which was prepared daily so that each millilitre contained 10 μg of scopolamine hydrobromide, ingested by each doe was recorded and the mean dose for the 5-day period calculated.

The treated does were killed on the 22nd day of gestation and their uteri examined to record the placement of live, dead or resorbed foetuses, as well as the number of implantation sites and corpora lutea. The eight control does, which received no medication, were allowed to deliver at term. The foetuses in the experimental group were examined for obvious malformations of the skull, body wall and limbs. They were then sectioned to inspect the brain, palate, thoracic and abdominal organs.

Results

The injection of scopolamine hydrobromide into the yolk sac of chick embryos after incubation for 96 h resulted in embryos having gastroschisis (Fig. 1), reduction deformities of the limbs (Fig. 2), and microphthalmia (Table 1), there being a significant difference between the groups receiving the drug and the controls in the number of embryo deaths ($\chi^2 = 35 \cdot 45$; $P < 0 \cdot 001$). Specific contrasts using a simultaneous confidence-interval procedure, with the probability of a type 1 error fixed at 0·05, were conducted to investigate this. Although there was a significant difference in the number of deaths between the control group and the combined treatment groups, this was not the case when the treatment groups were compared with each other, suggesting that this effect may not be dose-related. However, comparison of the incidence of deformities across the three groups ($\chi^2 = 89 \cdot 39$, $P < 0 \cdot 001$) and subsequent multiple comparisons suggest that the incidence of gastroschisis, in particular, is dose-related and this may also be the case for the other kinds of deformities noted.



Figs 1 and 2. Chick embryo with gastroschisis and beak deformity (Fig. 1) and with reduction deformity of the right leg and buphthalmia (Fig. 2) following injection of 200 μg scopolamine hydrobromide into the yolk sac after incubation for 96 h.

Figs 3 and 4. Rabbit foetus showing microphthalmia and hydrocephalus (Fig. 3) and with exencephaly and buphthalmia (Fig. 4). Doe ingested 473 μg scopolamine hydrobromide per kilogram body weight daily from the 10th to the 14th day of gestation.

In the second part of the experiment, two of the eight does given mean doses of 473 and 520 μg/kg of scopolamine hydrobromide orally had litters of deformed kittens (Table 2). In the group of eight does given the drug by intramuscular

Table 1. Effects of the injection of scopolamine hydrobromide into 100 White Leghorn eggs at 96 h incubation

Percentage incidence of deformity given in parentheses

Amount of drug injected	No. of embryos living at 12th day	Total No. of embryos deformed	No. of embryos with: Gastroschisis	Reduction deformity of leg	Wing deformities	Microphthalmia
100 μg	62	21 (34%)	21 (34%)	0	0	2 (3%)
200 μg	46	36 (78%)	36 (78%)	2 (4%)	4 (9%)	8 (18%)
Control[A]	86	0	0	0	0	0

[A] 400 μl isotonic saline injected.

Table 2. Effects of scopolamine hydrobromide administered to New Zealand White rabbit does on the 10–14th day of gestation

Eight control does produced 54 normal kittens

Doe No.	Weight (kg)	Daily dose (μg/kg)	No. of living foetuses	No. deformed	No. of resportions
		Intramuscular injection[A]			
1	2·73	74	9	0	0
2	2·68	74	5	0	0
3	3·64	110	6	0	1
4	3·03	132	6	0	2
5	3·72	216	9	0	1
6	2·92	274[B]	—	—	—
7	3·25	370[B]	—	—	—
8	4·03	298	3	0	3
Total			38	0	7
		Oral ingestion			
9	2·64	495	7	0	0
10	2·62	513	6	0	0
11	4·02	504	6	0	0
12	2·99	582	5	0	0
13	3·37	473	8	8	1
14	2·77	424	7	0	0
15	3·42	520	4	4	2
16	2·96	483	5	0	1
Total			48	12	4
Overall totals			86	12	11

[A] Injections divided into two equal doses and given at 12-hourly intervals (8 a.m. and 8 p.m.).
[B] Lethal.

injection, twelfth hourly from the 10th to the 14th day inclusive, in doses ranging from 37 to 185 μg/kg, two does died. The fatal doses were 137 and 185 μg/kg and death was due to the effect of the drug on the heart.

The deformities consisted of either exencephaly (1 doe, 8%), hydrocephaly (1, 8%), buphthalmia (2, 17%), or microphthalmia (10, 83%) but there was no evidence of gastroschisis or limb reduction deformities (Figs 3 and 4). The incidence of deformities was no greater in the group receiving the bolus injection of scopolamine hydrobromide than in the controls, whereas there was a significant difference ($\chi^2 = 25{\cdot}19$, $P < 0{\cdot}001$) in this incidence between the group receiving the drug orally and the control group. The daily dose ingested per kilogram body weight was from 1·5 to 7 times greater than that given by injection.

There was no significant difference in the incidence of resorptions between the groups in which the drug was administered orally or by injection whereas there was a significant difference ($\chi^2 = 8{\cdot}80$, $P < 0{\cdot}025$) in this incidence between the control groups and the combined treatment groups.

Discussion

Zacks (1954) demonstrated the presence of acetylcholinesterase in the stages of development of the embryonic chick from 0 to 96 h. Nachmansohn (1939) determined acetylcholinesterase activity in chick nerve and muscle at 6 or 9 days of age. He demonstrated the increasing levels of activity of this enzyme to about the 15th day and a higher rate of increase until hatching. It has been shown that the acetylcholinesterase inhibitor physostigmine will produce skeletal and limb deformities in the chick (Bueker and Platner 1956). Also the cholinomimetic drugs will produce a consistent syndrome of developmental defects comprising brevicollis and muscular hypoplasia of the legs (Landauer 1977). It is thought that in the case of the latter drugs, the effects are produced by the inactivation of acetylcholinesterase.

When scopolamine hydrobromide was injected into hen eggs after they had been incubated for 96 h it produced microphthalmia, buphthalmia, gastroschisis, exencephaly and reduction deformities of the limbs. In the experiment with rabbits, 2 of 14 litters (14%) were malformed. However, a daily dose of scopolamine hydrobromide in excess of 450 μg per kilogram body weight was required to produce these malformations. It was only possible to give massive doses orally (by administration in the drinking water), as bolus injection caused 25% mortality.

The experiment has shown that the administration of scopolamine hydrobromide in the early stages of development in one species of bird and one species of mammal will interfere with development, particularly of the eye and brain, in a significant percentage of embryos. It appears that the drug acts by interference with the function of the muscarinic receptors of the cholinergic nerves.

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After the above paper had been published it was discovered that because of difficulty in measuring rabbit water intake accurately with the containers used in the experiment described in that paper the amount of scopolamine hydrobromide ingested by the rabbits was less than as stated. It was therefore decided to repeat the experiment using a different type of water container. The original experiment was carried out in winter and the latter in summer and this could be another factor in the increase in water intake by the does in the present experiment.

Materials and Methods

Ten virgin New Zealand white rabbit does (Tillside Rabbits, Bargo, N.S.W.) weighing between 3·071 and 3·893 kg were mated with a healthy male. Copulation was observed and the day of mating was designated gestation day 1. The mated females were housed individually in polyvinyl-chloride–coated wire cages and were provided with rabbit chow (Doust and Rabbidge, Concord West, N.S.W.) and water *ad libitum*. The water used throughout the experiment was from the Sydney City Water Supply which is known not to contain teratogens.

The does were given scopolamine hydrobromide in the drinking water from the 10th to the 14th day of gestation inclusive. The amount of drinking water, which was prepared daily so that 1 ml contained 10 μg of scopolamine hydrobromide, ingested by each doe was recorded and the mean dose for the 5-day period calculated.

The treated does were killed on the 28th day of gestation and the uteri examined to record the placement of live, dead or resorbed foetuses as well as the number of implantation sites and corpora lutea.

Results and Discussion

Four of the does were found not to be pregnant (Table 1). The foetuses in the experiment were examined for obvious malformations of the skull, body wall and limbs. They were then sectioned to inspect the brain, eye, palate, thoracic and abdominal organs. In all, six pregnant does produced 38 foetuses. The 38 foetuses all exhibited either microphthalmia (34, 89·5%) or buphthalmia (4, 10·5%).

The method of oral administration of scopolamine hydrobromide in the drinking water makes it impossible to measure the exact dose of drug ingested by the doe, as some wastage inevitably occurs owing to movement of the doe in the cage and to the failure of the valve in the drinking tube to cut off the flow of water without some

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leakage after the doe ceases to drink. If the cages were left empty it was found that approximately 2 ml of water per day leaked from the bottles. However, the maximum possible dosage of scopolamine hydrobromide ingested by the doe is shown in this

Table 1. Effects of scopolamine hydrobromide administered to New Zealand White rabbit does on the 10th to the 14th day of gestation

Controls were as in the original experiment

Doe No.	Weight (kg)	Mean daily dose of scopolamine hydrobromide (μg/kg)	No. of living foetuses	No. with deformities	No. of resorptions
1	3·071	1114	5	5	3
2	3·692	579	10	10	0
3	3·825	444	7	7	0
4	3·893	893	6	6	0
5	3·590	929	0	0	0
6	3·263	658	0	0	0
7	3·460	561	0	0	0
8	3·775	572	6	6	0
9	3·372	914	4	4	0
10	3·222	750	0	0	0

experiment which confirms that massive doses of this drug, when given in the drinking water from the 10th to the 14th day of gestation, will produce malformations, particularly in the eye, in the New Zealand white rabbit foetus.

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