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Effects of Carbaryl on Gonadal Development in the Chick Embryo

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Carbaryl (1-naphthyl-N-methylcarbamate) is a member of the carbamate group of insecticides, which exhibit their insecticidal power by inhibiting cholinesterase enzymes (O'Brien, 1963). Although numerous studies have examined the effects of carbaryl on many species (Carpenter et al., 1961; Robens, 1969; Swartz, 1981) very few of these have been directed toward specific effects of this pesticide on the reproductive system. Those that have appeared have been of a conflicting nature.

Carbaryl at a dose level of 10,000 ppm impaired fertility in rats in that no litters were produced for the second mating of the second generation (Collins et al., 1971). A contrasting report showed carbaryl to be without effect on fertility or gestation in the rat (Weil et al, 1972).

In view of these rather inconsistent findings, it appeared necessary, not to immediately further examine the effect of carbaryl on the reproductive system of the adult organism, but rather to ascertain the role of this agent on critical stages in the embryological development of this system during the period of primordial germ cell (PGC) migration and mid-way in embryonic development following sex differentiation.

The orderly migration of PGCs from their extra-embryonic origin in all vertebrates to the developing gonadal anlagen is essential to insure the future fertility of the species. These PGCs are the sole source of the definitive sex cells. Any alteration in the migration of these cells and their subsequent colonization of the gonads would seriously hamper the normal reproductive activity of these animals.

In this study, chick embryos will be exposed to carbaryl prior to incubation. They will be examined after 5 days of incubation to assess PGC migration and colonization. In addition, this study will also examine the status of both male and female gonads near the mid-embryonic stage (12 days of incubation) to determine whether any adverse effects on the development and sexual differentiation of the gonad can be induced following a longer term exposure to carbaryl.

MATERIALS AND METHODS

Fertile white Leghorn eggs (Truslow Farms, Inc., Chestertown, Md.) were used in this study. Eggs were randomly divided into two groups - one receiving 10 mg carbaryl (technical grade, 99%) dissolved in acetone and the other the acetone vehicle only. All injections were made into the yolk sac and were carried out prior to incubation.

Eggs were removed from the incubator at two different times - at 5 days (Stages 24-27; Hamburger and Hamilton, 1951) and at 12 days of incubation (Stages 38-39). Embryos were removed and fixed in cold Gendre's fluid for 48 hours. Following fixation, the embryos were dehydrated, embedded in paraffin and serially sectioned at 8u and stained with the periodic acid-Schiff (PAS) technique.

In the 5-day embryos the primordial germ cells located both in the gonads and adjacent dorsal mesentery were counted and the means calculated. The student t test was employed to examine the difference between the number of PGCs found in carbaryl-exposed embryos and acetone-controls. In addition, the mitotic activity of the PGCs was also recorded to note any alteration in this cellular activity.

The 12-day ovaries and testes were examined histologically to determine whether any significant alterations occurred in the general structure of the gonads or in the cellular components of these organs that might predispose the organism to subsequent defects in fertility as an adult.

RESULTS AND DISCUSSION

The number of PGCs found in the embryos exposed to 10 mg of carbaryl for 5 days ranged from 382 to 1568 with a mean of 988.8 $\pm$ 439.4 S.D. (Table 1). Those exposed to only the acetone vehicle contained PGCs ranging in number from 500 to 1987 with a mean of 1284.3 $\pm$ 501.2. Although there was a decrease in the number of PGCs in the gonadal area of embryos exposed to carbaryl for 5 days, this decrease was not significant ($P>0.05$). Similarly, the exposure to carbaryl had little effect on the mitotic activity of the PGCs (Table 1). Although the percent of PGCs undergoing mitosis in carbaryl treated embryos was less than that of control embryos it was not significant ($P>0.1$).

The histology of the gonads and PGCs was not altered in any way. The PGCs of both groups reacted with the same intensity when stained with the PAS technique. The gonads were of normal size for this stage of development.

Embryos exposed to the pre-incubation injection of carbaryl for 12 days survived the exposure very well. Viewing the gonads macroscopically revealed seven male embryos and four females. The acetone control group contained sixteen males and eight females. The gonads appeared normal externally when compared to controls.

Table 1. Bilateral distribution and mitotic activity of primordial germ cells in the gonadal area of 5-day chick embryos following exposure to carbaryl.

Treatment	Embryo	Stage	Primordial Germ Cells				
			Total*	Left	Right	Dividing	% Dividing
Carbaryl	1	26	1566	1190	329	29	1.8
	2	26	382	275	105	5	1.3
	3	26	1138	1011	116	12	1.0
	4	25	618	452	149	0	0.0
	5	25	682	522	155	3	0.4
	6	26	1568	1291	246	18	1.2
	7	26	804	675	123	1	0.1
	8	26	1152	856	286	4	0.3
Acetone	9	25	1306	1091	194	8	0.6
	10	25	978	686	270	3	0.3
	11	25	864	706	139	22	2.5
	12	25	1746	1398	311	27	1.5
	13	26	1684	1224	435	18	1.1
	14	25	1987	1632	294	45	2.3
	15	26	1209	995	199	33	2.7
	16	25	500	344	124	8	1.6

*Includes mid-line PGCs to which no laterality could be ascribed.

Histologically, the gonads from treated embryos differed little from those of control gonads. The testes of carbaryl-exposed embryos contained primary sex cords containing numerous spermatogonia (derivatives of the PGCs). No pyknotic germ cells were found in either group. The ovaries of the pesticide-exposed females exhibited the normal asymmetry in size with the left one being much larger. The secondary sex cords were present and the left gonad contained numerous dilated medullary cords.

Carbaryl has been implicated as being deleterious to the reproductive system (Collins et al., 1971; Shtenberg and Ozhovan, 1971). The study presented here was designed to determine whether carbaryl-induced effects on the reproductive status of the adult gonads might, in fact, have their bases in a disturbance of the embryological development of these organs, which effect has been demonstrated by other pesticides.

In embryos exposed to carbaryl for 5 days, the mean number of PGCs in the gonadal area was lower than that of controls, but this difference was not statistically significant. This differed from the reports of investigators (David, 1973; Bruel and David, 1981) who, working with DDT and dichlorvos, organochlorine insecticides, demonstrated a significant decrease in the number of PGCs colonizing the gonads. In addition, they observed degeneration of numerous germ and somatic cells, pyknotic hypertrophy of several PGCs and a decrease in their mitotic activity. In the present study there was neither any indication of degenerative changes in either the PGCs or the somatic cells of the developing gonads nor was there any alteration in the mitotic activity of the PGCs at this time. Exactly, how the organochlorines exert this effect is not known. It has been suggested that dichlorvos disturbs the surface and matrical glycoconjugates of tissues involved in PGC migration, thereby, altering the normal migration of these cells (Bruel and David, 1981). Carbaryl is not known to possess this capacity.

Embryos exposed to carbaryl for 12 days exhibited no alterations in testicular or ovarian morphology. In addition, there was no predisposition with respect to sex of the exposed embryos.

Data from this study indicate that carbaryl does not elicit the deleterious effects on the developing avian gonads as has been demonstrated by some of the organochlorine pesticides. It may be that the germ cells in this early stage of development are insensitive to the actions of carbaryl and only acquire susceptibility to this agent later in life, since an increase in the frequency of abnormal spermatozoa and a reduction in the number of spermatogonia and spermatozoa have been reported in adult mice exposed to carbaryl (Degraeve et al., 1976; Kitagawa et al., 1977).

On the other hand, carbaryl may be readily metabolized within the yolk-embryo milieu similar to its rapid metabolism in the adult vertebrate (Kuhr and Dorough, 1976). The allantois is the excretory organ of the chick embryo. It is here that the

respiratory interchange of CO_2 and O_2 occur. This organ also serves as a reservoir for the excretions emanating from the developing excretory organs. Further studies examining the presence of carbaryl and/or its metabolites in the fluid of the allantois of the exposed embryo might help to clarify the fate of carbaryl in the chick embryo.

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