Effect of Dinitrophenol on Lens of Chick Embryo.* (24137)

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Horner(1) reported that therapeutic use of 2,4-dinitrophenol (DNP) for control of obesity had resulted in cataracts in a small number of patients. Robbins(2) produced cataracts in the chick by addition of 0.25% DNP to the diet. Occurrence of this lesion was not prevented by addition of riboflavin to the diet. Buschke(3) showed that a threshold dose of 0.06 mM of DNP/kilo body weight produced an opacity in the lens of the chick when administered either by stomach tube or intramuscularly. Bettman(4) produced cataracts in chicks and rabbits by direct injection of DNP into the anterior chamber of the eye. Rabbits, however, did not develop cataracts when fed DNP. This study was initiated to determine the effect of DNP on the lens of the chick embryo.

Method. White Leghorn X New Hampshire eggs were injected with a solution of DNP in physiological saline prior to incubation and on fifth, eighth and fifteenth day of the 21-day incubation period. A small area over the air cell of the egg was painted with tincture of merthiolate and a small hole drilled through the shell. A 23 gauge needle was used for injection of DNP solution. The hole was then sealed with collodion after injection. The eggs were incubated in a standard commercial incubator. The eggs were candled daily and dead embryos removed for gross study and an estimation of time of death. Embryos which failed to hatch were treated similarly. The eyes of live embryos and chicks that hatched were examined for lenticular changes with a Bausch and Lomb Ophthalmoscope equipped with May-type optics. The number of embryos, amount of DNP and time of injection are shown in Tables I and II.

Results. Eighty eggs were injected with 1-25 μg of DNP prior to incubation (Table I). The mortality was high on third day of incubation and progressively increased with increasing concentrations of DNP injected. Surviving embryos appeared normal when hatched. The live-unhatched embryos exhibited an edematous area at the back of the neck similar to that described by Ferguson *et al.*(5) in Vit. E-deficient turkey embryos. No lens changes were observed in the chicks or live-unhatched embryos. There is a normal mortality peak at the third day of incubation of chicken eggs(6). This peak is amplified in deficiencies of Vit. E, biotin or riboflavin. Needham(7) advanced evidence suggesting a change in oxidative metabolism of carbohydrates occurring at third day which may be responsible for the normal mortality peak. It is possible that DNP interferes with this change.

One hundred and seventy-three fertile eggs were injected with 25-200 μg of DNP on the fifth day of incubation (Table II). Embryonic mortality after injection increased with increasing levels of DNP employed with all embryos killed by the 200 μg level (Table II). Cataracts were not observed in samples examined 20 hours after injection or embryos examined on 19th day.

TABLE I. Effect of Injecting DNP Prior to Incubation, 20 Eggs/Series.

Treatment	No. of fertile eggs	No. dead at 3 days	No. chicks hatched	% hatch of fertile eggs
Uninj.	18	1	17	94.4
Drilled, uninj.	18	2	14	77.8
.1 ml physiol. saline	18	3	12	66.7
1 μg DNP	17	10	5	29.4
5 " "	15	10	4	26.7
10 " "	16	11	3	18.8
25 " "	14	11	1	7.1

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TABLE II. Effect of DNP Injected after 5, 8 and 15 Days of Incubation.

Day of inj.	Treatment, μg DNP	No. fertile eggs	20 hr after inj. No. removed for observation	20 hr after inj. No. with cataracts	20 hr after inj. No. dead embryos	Unhatched embryos No.	Unhatched embryos No. with cataracts*	Hatched chicks No.	Hatched chicks No. with cataracts*
5	Saline control	19	4	0	1	1	0	13	0
	25	35	4	0	4	4	0	23	0
	30	14	4	0	9				
	40	14	4	0	9				
	50	14	4	0	7				
	60	14	4	0	7				
	70	14	4	0	10				
	80	14	4	0	9				
	100	29	6	0	22				
	200	25	5	0	20				
8	Saline control	20	6	0	1	2	0	11	0
	25	48	6	0	2	2	0	38	0
	100	36	6	0	1	5	0	24	0
	200	20	8	8	2	2	1	8	3
	300	20	6	6	8	2	2	4	2
	400	20	6	6	11	1	1	2	1
	500	20	6	6	8	2	2	4	4
15	Saline control	20	4	0	0	1	0	15	0
	100	30	5	0	1	3	0	21	0
	750	47	5	5	20†	2	2†	20	0

* Cataract in both eyes.
† Opacities observed in all cases may have been post-mortem changes.

One hundred sixty-four eggs were injected with 25-500 μg DNP after 8 days incubation. Cataracts were produced only in embryos from groups injected with 200-500 μg (Table II). Approximately one-third of the lens area was opaque when viewed from the anterior aspect. Opacity was situated in the center of the lens and proceeded posteriorly to the central cortical area. Seven live embryos which failed to hatch had cataracts and exhibited an extensive edematous area of gelatinous nature at back of neck, not observed in the control groups. This condition is similar to that described for Vit. E-deficient turkey poults(5). In a few cases the edema was so extensive that the vertebrae could be easily seen. A crude measurement was made of the cataract with the grid system of the ophthalmoscope for comparison with opacity observed in hatched chicks. The opaque areas for lenses of 9-day embryos were found to be approximately the same size regardless of dosage of DNP employed. Opacities in hatched chicks injected with 500 μg of DNP were approximately the same size and of the same nature as 9-day embryos. However, with smaller doses of DNP the size of cataracts in the hatched chick was smaller than that observed in 9-day embryos. This suggests that the DNP had no further cataractogenic action after the initial opacity was produced. This also suggests that some regression of opacity occurred with diminishing dosage of DNP.

DNP injections failed to produce a cataract when injected on fifth day. At this time the proximal wall of embryonic lens sac approaches the distal wall(10). The latter becomes the anterior marginal epithelium of the

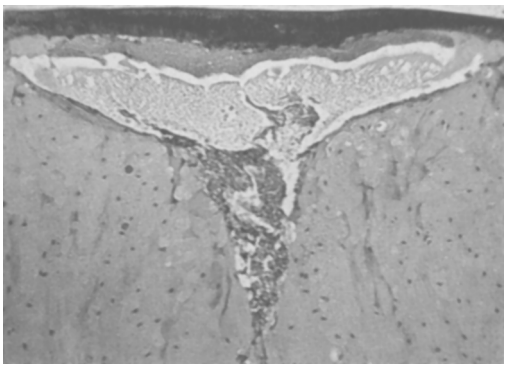

FIG. 1. Degenerative area in microscopic section through lens of hatched chick inj. with 500 μg of DNP on 8th day of incubation. (Hematoxylin and eosin) 58×.

lens. The cataract formed when DNP was injected on 8th day of incubation occurred when the 2 walls were apparently in contact (10). The oxidative enzyme systems are found only in epithelium, and the cortex is dependent on the epithelium for its metabolism(2.9). It is possible that this relationship may be involved in cataract formation.

Several hatched chicks were sacrificed for histological studies. Fig. 1 shows the lens of a newly hatched chick which received 500 μg of DNP on 8th day of incubation. There is a degeneration of lens fibers and a liquefaction of lens protein. This degeneration in the lens of the chick is similar to that previously observed in Vit. E-deficient turkey embryos(5,11).

Seventy-seven eggs were injected with 100 and 750 μg of DNP after 15 days incubation. The 100 μg level had no effect. The 750 μg level resulted in lens opacities in embryos from 20 hour post-injection sample and live-unhatched embryos. Hatched chicks appeared normal.

Summary. 1) Injections of 1-25 μg of DNP into chicken eggs, prior to incubation resulted in embryonic mortality after 3 days incubation, but did not produce lens changes. 2) Injections of 25-200 μg of DNP after 5 days incubation did not produce lenticular changes. 3) Cataracts were produced in embryos and hatched chicks from eggs injected with 200-500 μg of DNP after 8 days incubation. The size of cataract was roughly the same in hatched chicks as in embryos from the 500 μg group. 4) Injection of 750 μg of DNP after 15 days incubation produced an opacity evident after 20 hours. However, the opacity apparently regressed since the eyes of chicks hatched from this group appeared to be normal.

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Effect of Chlorpromazine on Swimming Time of Rats at Different Temperatures. (24138)

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Recently an analysis was made of the factors responsible for the marked hypothermia observed in rats treated with chlorpromazine (1). Chlorpromazine has also been shown to induce some muscle paralysis in rats(2). We have therefore studied the effect of chlorpromazine on swimming time of rats in water bath at different temperatures.

Methods. Male albino rats weighing 200 g were used. Swimming time of control and chlorpromazine-treated groups, composed of 6 rats, was measured when animals were swimming at 9, 19, 28 or 32°C. Chlorpromazine† 10 mg/kg was injected intraperitoneally 5 minutes prior to beginning of swimming and the rectal temperature was recorded with thermocouples. Room temperature was maintained at 21°C and water bath remained at

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