

## PILOT STUDIES WITH PROCAINHYDROCHLORIDE AND THE DEVELOPING CHICK EMBRYO<sup>1</sup>

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While studying the effects of irradiation on avian sperm and the developing chick embryo, the senior author became concerned about the influence of chemical radioprotectants. When on sabbatical leave from 1968-1969, several individual chemicals and combinations of chemicals were injected into embryonating eggs (Morgan *et al.* 1969). Potential reports of the use of procainhydrochloride as a protective agent, both in plants (Schwanitz and Schwanitz, 1968) and in animals (Schwanitz, 1966) prompted an investigation into the tolerance range of this chemical in chick embryos. This paper reports a dose by age tolerance range.

### MATERIALS AND METHODS

Eggs were used from individually caged Rhode Island Red and White Leghorn hens which were artificially inseminated with semen from Rhode Island Red cocks.

Trial I. One-hundred and twenty fertile eggs were used at three age levels: six-day embryo, three-day embryo and zero-day embryo (before incubation). Guided by concentrations which had been successfully used for plants and animals, we extended our range from supposed protective levels to supposed toxic levels. The concentrations of procainhydrochloride ( $C_{13}H_{21}ClN_2O_2$ ) employed were: 0.0 (control), .02, 0.2, 2.0 and 20 mg/ml. Following the procedure of Morgan *et al.* (1969) eight fertile eggs from each group were injected with 0.1 ml. of fluid into each air cell. The holes were then sealed with scotch tape and the eggs were returned to the incubator. At two-day intervals the eggs were candled to ascertain progressive embryonic development. When development had obviously ceased, the eggs were opened and contents were examined. Approximate age at death and embryonic lesions were recorded.

Trial II. A similar procedure was followed for the 400 eggs used for Trial II. The design for this trial involved chick embryos aged from zero through seven days and procaine concentrations of 0.0 (control), 2.0, 6.6, 20.0 and 66.6 mg/ml. Guided by preliminary results from Trial I, several of the age x treatment groups were omitted.

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For each of the trials, eggs with viable embryos were transferred to a hatching compartment after 18 days of incubation. Chicks were observed and recorded on the 21st and 22nd day. The contents of all eggs which did not hatch by the 22nd day were examined.

### RESULTS

In our first trial (Table 1) there was good hatchability for all of the concentrations except the high concentration. Twenty of 21 hatched in the control group and a combined total of 77 chicks hatched from 87 eggs in the three low concentration groups. However, only seven from 20 hatched in the 20 mg/ml. group.

Table 1. Trial I hatchability of eggs which were treated with 0.1 ml of procaine concentrations ranging from .02 to 20.0 mg/ml.

Embryo Age (days)	Control H(OH)	mg/ml of procainhydrochloride			
		.02	0.2	2.0	20.
0	5/6*	6/6	7/8	6/7	4/6
3	7/7	7/7	5/6	6/9	0/7
6	8/8	6/8	7/8	7/8	3/7

\*Numerator = hatched chicks  
Denominator = fertile eggs

Weak chicks were observed in the procaine injected groups, but not in the control group, where the eggs were injected with 0.1 ml. of water. In addition to high mortality in the 20 mg/ml. group, there was further evidence of the damaging influence of procaine in lower concentration. Five of the 19 chicks which hatched in the 2.0 mg/ml. group were weak. In all, seven of the 84 were classified as weak chicks. There was one weak chick in the 6-day group receiving a .02 mg/ml. injection and one in the 6-day group receiving a 0.2 mg/ml. injection.

Hatchability of controls in Trial II (Table 2) was poorer than for Trial I. This was probably due to a lower humidity in the incubator at hatching time. Consideration of hatchability percentages for all age groups combined is as follows: control—52%, 2.0 mg—47%, 6.6 mg—45%, 20 mg—28% and 66 mg—8%. The trend indicates a sharp reduction of hatchability at 20 and 66 mg/ml. concentrations. Indeed, none of the 3- and 4-day embryos hatched with the 20 mg/ml. dosages and none of the 5- and 6-day embryos survived the 66 mg/ml. dosages.

Table 2. Trial II hatchability of eggs which were treated with 0.1 ml of procaine concentrations from 2.0 to 66.0 mg/ml.

Embryo Age (days)	Control	mg/ml of procainhydrochloride			
		2.0	6.6	20.0	66.0
0	8/11*	---	6/13	3/10	---
1	7/15	10/15	6/13	4/12	---
2	4/13	6/15	2/17	2/18	---
3	6/14	3/12	6/15	0/12	---
4	5/11	7/16	4/10	0/13	---
5	---	7/13	9/12	5/12	0/12
6	7/10	---	7/12	3/11	0/10
7	5/7	---	7/13	10/13	3/13

\*Numerator = hatched chicks  
Denominator = fertile eggs

There was no trial which involved both procaine treatment and embryo irradiation and no interaction between breed and treatment was observed.

### DISCUSSION

Since the work of Cheymol *et al.* (1961) several studies have been conducted with concern for the radioprotection provided by procaine. Preliminary studies with five radioprotective agents unrelated to procaine, prompted the authors to look for related anomalies when determining tolerance levels. As had been indicated earlier (Morgan, 1966) an age sensitivity is evident at varying concentration levels. Although weak chicks were observed in trial I, no other obvious deleterious effects, such as those observed in irradiated embryos (Morgan, 1968), were observed in the surviving chicks.

Age by treatment survival rate, as reported here, would suggest the tolerance range for future studies of radioprotection with procaine that is indicated in table 3. These data suggest that the two-day chick embryo may be the most vulnerable to procaine treatment. Further studies could clarify this point. In any event, young embryos prior to the seventh day were less tolerant than were the seven-day embryos. This pattern is similar to results from gamma irradiation treatments of the avian embryo (Morgan, 1966).

Table 3. Upper recommended concentration tolerance levels for procaine injections of embryos ranging from 0 to 7 days old. Older embryos are more tolerant.

Embryo Age (days)	Procaine Concentration (mg/ml)
0	6.6
1	6.6
2	2.0
3	6.6
4	6.6
5	6.6
6	6.6
7	20.0

### CONCLUSIONS

Chicken embryos ranging from 0 to 7 days old at time of treatment, were injected with concentrations of procainhydrochloride which ranged from .02 to 66 mg/ml. Only seven-day embryos survived the highest concentration, and not all of the chicks from this group were able to hatch. The 2.0 mg/ml. concentration permitted survival in each age group. The two-day embryos were least tolerant to procaine injections. A recommended dosage rate has been devised for the different age levels.

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