# *The Effect of Cyclopropane on Mitosis in Chicken Embryos*

*Sergei Snegireff, M.D.,* and Nikaan B. Andersen, M.D.†*

**Fertilized chicken eggs 24 to 72 hours old were incubated in cyclopropane 20–50 vol per cent for three to 12 hours. Zero to 48 hours after treatment the embryos were sacrificed and mitotic figures in the neural tube counted. Cyclopropane had two effects on cells in mitosis, increasing the ratio of cells in metaphase to cells in prophase, and decreasing the number of cells in mitosis. The former was seen primarily after exposure to low cyclopropane concentrations, the latter after high concentrations. Twenty-four hours after discontinuation of treatment the distribution of cells in mitosis appeared normal. (Key words: Chicken; Embryo; Mitosis; Cyclopropane.)**

FOR NEARLY A CENTURY, anesthetics have been known to affect cell division,[1] an effect recently extensively reviewed.[2] The antimitotic effect is assumed to be partly a reversible dissolution of the mitotic spindle with delay in the metaphase ("C-mitosis"),[3, 4] and partly an inhibition at a point prior to normal initiation of mitosis,[5, 6] perhaps at DNA synthesis.[7] In our opinion these two possibilities need not be be mutually exclusive.[8]

Studies of chicken[9, 10] and rat embryos[11] published during the last five years show that anesthetics may have teratogenic potential as well. Demonstration of the teratogenic effect in an aplacentary species such as the chicken suggests a direct effect on the developing embryo. Cyclopropane was found to have a lethal effect as well as a teratogenic effect on chicken embryos at concentrations considered anesthetic for the species.[10]

It is tempting to assume a connection between antimitotic and teratogenic effects. However, no such relationship has been established for anesthetics. We doubt that it is possible to prove or disprove a cause–effect association at this time. Nevertheless, we hypothesized: 1) that cyclopropane, like other anesthetics, has antimitotic effects; 2) that if intra- as well as premitotic effects are present, a study of appropriate design would clarify this; 3) that the chicken embryo might be a useful model, since it is known to be sensitive to the teratogenic effects of cyclopropane.[12] We tested these hypotheses, and the results are reported here.

## Method

Two hundred and fifty fertilized eggs from white Leghorn chickens obtained from a commercial source were incubated in a 2,000-egg incubator at 37.5 C and 65 per cent relative humidity. Incubation was started on the morning of the day counted as day one of incubation. Test eggs were treated in a 200-egg treatment incubator in an atmosphere of 20 per cent oxygen, various cyclopropane concentrations, and nitrogen. Oxygen concentration was maintained at 21 ± 2.0 per cent with the aid of a Beckman D2 oxygen analyzer. Cyclopropane was delivered from an anesthesia machine, the concentration in the incubator monitored by gas chromatography and maintained within ±5 per cent of the desired level. Inflow and venting of gases were sufficient to maintain near-normal carbon dioxide concentrations. For each group of test embryos treated with cyclopropane, a similar group was treated with air only, serving as the control.

We tested the effects of four variables: 1) cyclopropane concentration; 2) duration of treatment; 3) embryonic age at treatment; 4)

* Assistant Professor.
† Professor.
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Requests for reprints should be addressed to Dr. Andersen.

TABLE 1. Effects of Cyclopropane on Chicken Embryos Sacrificed 12 Hours after Treatment (Means ± 1 Standard Error of at Least Ten Embryos)

| Cyclopropane (Vol Per Cent) | Duration of Treatment (Hours) | Age of Embryos (Hours) | Prophase in Per Cent of Mitoses | P | Metaphase in Per Cent of Mitoses | P | $I_m$, Mitoses in Per Cent of Cells | P |
|---|---|---|---|---|---|---|---|---|
| 0 | 12 | 36 | 56 ± 8.7 | <.01 | 31 ± 7.6 | <.01 | 4.07 ± 0.71 | — |
| 20 | | | 41 ± 4.6 | | 42 ± 1.9 | | 4.61 ± 0.40 | |
| 0 | 12 | 36 | 56 ± 8.7 | — | 31 ± 7.6 | — | 4.07 ± 0.71 | <.01 |
| 35 | | | 54 ± 4.4 | | 35 ± 2.7 | | 2.88 ± 0.66 | |
| 0 | 12 | 36 | 56 ± 8.7 | — | 31 ± 7.6 | — | 4.07 ± 0.71 | <.01 |
| 50 | | | 59 ± 8.4 | | 32 ± 7.6 | | 2.69 ± 0.59 | |

time from end of treatment to sacrifice. The embryos were treated according to the following:

I) 36-hour-old embryos received 20, 35, or 50 vol per cent cyclopropane for 12 hours, then were sacrificed 12 hours after treatment.

II) 36-hour-old embryos received 50 vol per cent cyclopropane for three, six, or 12 hours, then were sacrificed 12 hours after treatment.

III) 24-, 36-, or 72-hour-old embryos received 50 vol per cent cyclopropane for six hours, then were sacrificed 12 hours after treatment.

IV) 36- or 72-hour-old embryos received 50 vol per cent cyclopropane for six hours, then were sacrificed 0, 12, 36, or 48 hours after treatment.

Fifteen eggs were in each treatment group. After treatment the embryos were either sacrificed or returned to the larger incubator for the appropriate time before sacrifice. At sacrifice the embryos were removed from the shells and placed in buffered saline solution, fixed in Bouin's fluid, embedded in paraffin and serially sectioned at 5 $\mu$. The sections were mounted and stained by a standard technique.[6]

Satisfactory sections were examined under oil immersion (1,000×), all counting limited to the neural tube area. Mitotic index ($I_m$) was determined as the percentage of mitotic figures in all cells present. The number of cells in each phase of mitosis and the total

TABLE 2. Effects of Cyclopropane for Different Lengths of Time on Chicken Embryos Sacrificed 12 Hours after Treatment (Means ± 1 Standard Error of at Least Ten Embryos)

| Cyclopropane (Vol Per Cent) | Duration of Treatment (Hours) | Age of Embryos (Hours) | Prophase in Per Cent of Mitoses | P | Metaphase in Per Cent of Mitoses | P | $I_m$, Mitoses in Per Cent of Cells | P |
|---|---|---|---|---|---|---|---|---|
| 0 | 3 | 36 | 54 ± 6.6 | — | 34 ± 5.4 | — | 3.44 ± 0.89 | — |
| 50 | | | 50 ± 4.2 | | 35 ± 4.8 | | 3.25 ± 0.93 | |
| 0 | 6 | 36 | 60 ± 8.8 | <.01 | 30 ± 3.5 | <.01 | 4.01 ± 0.85 | <.05 |
| 50 | | | 46 ± 1.1 | | 44 ± 3.9 | | 3.29 ± 0.36 | |
| 0 | 12 | 36 | 56 ± 8.7 | — | 31 ± 7.6 | — | 4.07 ± 0.71 | <.01 |
| 50 | | | 59 ± 8.4 | | 32 ± 7.6 | | 2.69 ± 0.59 | |

neural tube cell population were also recorded. Mitotic index was assessed on the basis of the count in at least 1,000 cells per embryo. The mitotic index and each mitotic phase were recorded as the mean ±1 standard error in at least ten embryos for each treatment or control group. Cells totaling 400,000 were counted from the neural tubes of 240 embryos. Because of differences in absolute numbers for each phase of mitosis, cells in prophase and cells in metaphase were expressed as the percentages of all mitotic figures present. Large scatters in the results of anaphase and telophase counts precluded statistically significant data and meaningful information, and these counts were not included.

In each instance, the results in the test embryos were compared with the results found in equal numbers of nontreated control embryos. Student's *t* test for unpaired data was used for statistical evaluation of the results.

## Results

Treatment of 24–48-hour-old chicken embryos with cyclopropane was associated with significant changes in the distribution of cells between mitosis and the intermitotic phase, *i.e.*, $I_m$, and the distribution between different mitotic phases, *e.g.*, prophase and metaphase. Table 1 shows the effects of different cyclopropane concentrations at 12 hours in 36-hour-old embryos. Twenty per cent cyclopropane decreased the relative number of cells in prophase and increased the number of metaphase. There was no effect on $I_m$. Thirty-five and 50 per cent cyclopropane did not alter the distribution in mitosis, but decreased the total number of cells in mitosis ($I_m$).

In table 2 it is seen that variation in duration of treatment with cyclopropane, 50 per cent, under conditions similar to those in table 1 had effects comparable to those seen after changes in cyclopropane concentration. Three hours of treatment had no effect, six hours yielded a decrease in cells in prophase, an increase in cells in metaphase and a small decrease in $I_m$, while 12 hours decreased $I_m$ only.

The response to treatment for six hours with cyclopropane, 50 per cent (table 3), was affected only slightly by the age of the embryo at the start of treatment. In all instances except one the responses were the same: a decrease in cells in prophase, an increase in cells in metaphase, and a decrease in $I_m$. The exception was a lack of effect on metaphase in the youngest embryos.

All of the embryos described thus far were sacrificed 12 hours after discontinuation of treatment. On the average, the greatest response was seen when normal incubation was allowed to continue for this period of time after intervention. Table 4 shows that the effect of cyclopropane, 50 per cent, for six hours in 36- or 72-hour-old embryos had all but disappeared 24 hours after treatment was stopped; the only residual effect was a decrease in $I_m$ in the 72-hour-old embryos. Forty-eight hours after treatment no differences between mitotic counts in test and control embryos were seen.

TABLE 3. Effect of Age on the Responses to Cyclopropane of Chicken Embryos Sacrificed 12 Hours after Treatment
(Means ± 1 Standard Error of at Least Ten Embryos)

| Cyclopropane (Vol Per Cent) | Duration of Treatment (Hours) | Age of Embryos (Hours) | Prophase in Per Cent of Mitoses | *P* | Metaphase in Per Cent of Mitoses | *P* | $I_m$, Mitoses in Per Cent Of Cells | *P* |
|---|---|---|---|---|---|---|---|---|
| 0 | 6 | 24 | 60 ± 2.2 | <.01 | 31 ± 4.4 | — | 4.53 ± 0.40 | <.01 |
| 50 | | | 55 ± 3.4 | | 31 ± 2.5 | | 3.37 ± 0.48 | |
| 0 | 6 | 36 | 60 ± 8.8 | <.01 | 30 ± 3.5 | <.01 | 4.01 ± 0.85 | <.05 |
| 50 | | | 46 ± 1.1 | | 44 ± 3.9 | | 3.29 ± 0.36 | |
| 0 | 6 | 72 | 53 ± 3.1 | <.01 | 35 ± 4.9 | <.01 | 3.60 ± 0.77 | <.01 |
| 50 | | | 41 ± 4.4 | | 48 ± 3.6 | | 2.36 ± 0.76 | |

TABLE 4. Duration of Cyclopropane Effects in Chicken Embryos (Means ± 1 Standard Error of at Least Ten Embryos)

| Cyclopropane (Vol Per Cent) | Duration of Treatment (Hours) | Age of Embryos (Hours) | Time of Sacrifice after Treatment (Hours) | Prophase in Per Cent of Mitoses | P | Metaphase in Per Cent of Mitoses | P | Im. Mitoses in Per Cent of Cells | P |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 6 | 36 | 0 | 54 ± 2.7 | <.01 | 31 ± 3.5 | <.01 | 4.30 ± 3.45 | <.01 |
| 50 | | | | 43 ± 4.2 | | 45 ± 4.7 | | 3.46 ± 0.37 | |
| 0 | 6 | 36 | 12 | 60 ± 8.8 | <.01 | 30 ± 3.5 | <.01 | 4.01 ± 0.85 | <.05 |
| 50 | | | | 46 ± 1.1 | | 44 ± 3.9 | | 3.29 ± 0.36 | |
| 0 | 6 | 36 | 24 | 52 ± 3.9 | — | 40 ± 5.7 | — | 2.45 ± 0.80 | — |
| 50 | | | | 51 ± 6.6 | | 41 ± 6.9 | | 2.39 ± 0.79 | |
| 0 | 6 | 36 | 48 | 59 ± 4.6 | — | 30 ± 3.8 | — | 1.86 ± 0.30 | — |
| 50 | | | | 58 ± 3.9 | | 31 ± 5.0 | | 1.81 ± 0.35 | |
| 0 | 6 | 72 | 0 | 56 ± 2.8 | <.01 | 33 ± 2.9 | <.01 | 5.75 ± 0.91 | <.01 |
| 50 | | | | 41 ± 5.0 | | 46 ± 3.5 | | 2.63 ± 0.24 | |
| 0 | 6 | 72 | 12 | 53 ± 3.1 | <.01 | 35 ± 4.9 | <.01 | 3.60 ± 0.77 | <.01 |
| 50 | | | | 41 ± 4.4 | | 48 ± 3.6 | | 2.36 ± 0.76 | |
| 0 | 6 | 72 | 24 | 54 ± 7.2 | — | 36 ± 5.6 | — | 2.43 ± 0.70 | <.05 |
| 50 | | | | 55 ± 3.9 | | 38 ± 5.2 | | 1.86 ± 0.42 | |

## Discussion

The fertilized egg can be viewed as a three-compartment model: white, embryo, and yolk. We previously estimated the tension of cyclopropane in the embryos to be about 50 per cent of the ambient tension three to five hours after initiation of treatment. These computations were based on solubility coefficients for cyclopropane in white and yolk and the estimated flow rate constants.[10] Based on these premises, we assume that treatment for three hours with cyclopropane 20 vol per cent was associated with a cyclopropane tension at the site of action in the embryo corresponding to 10 per cent of atmospheric pressure, and that after three hours the tension increased relatively slowly. The use of eggs as an experimental model excludes the possibility of indirect effects on the embryos through modification of maternal or placental functions. Previously, anesthetics were found to arrest growth reversibly in cell cultures.[12] Accordingly, the mitotic effect seen here may well have been the result of a direct cyclopropane effect on the neural tube cell mass at concentrations comparable to those found to be teratogenic or lethal in chicken embryos,[10] and comparable to the cyclopropane concentrations used for anesthesia in man.

No effects were seen in embryos sacrificed 72 hours after discontinuation of treatment. No difference in the results was found when the embryos were sacrificed within the first 12 hours after treatment. Since it must have taken at least six hours for most of the cyclopropane to diffuse from the eggs, mitotic effects can be assumed to be rapidly reversible. This does not exclude the possibility that effects on mitosis might have resulted in a deformed fetus, had the embryo not been sacrificed.

Cyclopropane was found to have two dis-

tinctively different effects on cells in mitosis: a) it increased the relative number of cells in metaphase and decreased the relative number in prophase; b) it decreased the number of cells in mitosis. We do not know whether these two effects are unrelated or are manifestations of one basic effect of cyclopropane on mitosis.

The effect on the distribution of cells in mitosis (the "metaphase" effect) was seen predominantly with smaller cyclopropane concentrations after shorter treatment and in older embryos. Thus, 20 per cent for 12 hours in 36-hour-old embryos decreased the ratio prophase:metaphase, while an increase in $I_m$ was suggested, a picture consistent with a block or delay of mitosis in the metaphase. Arrest in metaphase has been found in cultures of embryonic mouse cells after treatment with nitrous oxide.[3] We found this effect on metaphase to be reversible, but the technique used did not allow us to determine whether the spindle was primarily affected or whether the chromosomes were arranged in characteristic patterns. These features are pathognomonic for a well-known characteristic arrest of mitosis in the metaphase[14] first shown with colchicine ("C-mitosis"[15]) and later demonstrated with chloral hydrate in rabbits[16] and in plants exposed to ethyl ether, chloroform, or chloretone.[17] The metaphase effect seen with cyclopropane was compatible with, but was not proven to be "C-mitosis."

The effect on the total number of cells in mitosis (the "$I_m$" effect) was seen predominantly with higher cyclopropane concentrations after longer treatment. Thus, 50 per cent cyclopropane for 12 hours in 36-hour-old embryos decreased the mitotic index only. We interpret this to mean that the cells were prevented from entering mitosis, but we have no information permitting further localization of the site of action. This finding is compatible with an earlier report that anesthetics may inhibit DNA synthesis, thus blocking entrance into mitosis.[7] Many agents, including antimetabolic compounds such as urethane, are known to have this effect.[18]

As stated above, the metaphase effect was elicited in a relatively pure form with cyclopropane, 20 per cent, and the $I_m$ effect with cyclopropane, 50 per cent, in 36-hour-old embryos. In nearly all other treatment groups the two effects appeared to be present simultaneously. Treatment for three hours had no effect at all, suggesting that if treatment lasts three hours or less, cyclopropane concentrations sufficient to elicit an effect are not reached in the embryo. When, on the other hand, effective concentrations were maintained, both effects were always present, but in various degrees. Thus, a weak stimulus was associated primarily with the metaphase effect and a stimulus of increasing intensity gradually introduced the $I_m$ effect. A large $I_m$ effect overshadowed the metaphase effect and prevented its manifestation. Maybe no cells entered mitosis or moved between any of the mitotic phases in the presence of high cyclopropane concentrations.

Unfortunately, the ages of the embryos and the cyclopropane concentrations used in this study are not wholly comparable to those used in a previous study of cyclopropane teratogenicity in the chicken.[10] To the extent that comparison is possible, it appears that increasing intensity of the cyclopropane stimulus tends to favor death over deformation in 36–48-hour-old embryos.

We conclude that cyclopropane *in vivo* interferes with the normal division of embryonic neural tube cells in the chicken. Anesthetic cyclopropane concentrations partly delay mitosis in the metaphase and partly decrease the number of cells entering mitosis, both reversible effects. The former is seen mainly with low, the latter mainly with high, cyclopropane concentrations. These effects on mitosis may or may not be related to the teratogenic effect of cyclopropane on the chicken.

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## Drugs

**ATROPINE METABOLISM** Administration of two $^{11}$C-tropine-labeled atropines to man showed that 77 to 93 per cent of the injected dose was excreted in the urine in 24 hours. The N-methyl-$^{14}$C-atropine, but not the 2-4-$^{14}$C-atropine, showed oxidation to $^{14}CO_2$ and elimination through the lungs, the magnitude of excretion by this route amounting to 3 per cent of the drug in three hours. (*Kalser, S. C., and McLain, P. L.: Atropine Metabolism in Man, Clin. Pharmacol. Ther. 11: 214 (March) 1970.*)

**TRICHLOROETHYLENE** Analgesia was produced in 73 patients during the first week after abdominal operations by inhalation of 0.5 per cent vol trichloroethylene in air. Inhalation was continued for an average of two to three minutes, following which respiratory therapy was carried out. There were marked increases in vital capacity, forced expiratory volume, and deep respiratory movements, and an effective cough reflex developed. No complications were observed. (*Polaczek-Kornecki, T., Mroz, A., and Sokolowska, T.: The Use of Trichlorocthylene Analgesia in Postoperative Respiratory Gymnastics Following Upper Abdominal Operations, Der Anaesthesist 18: 410 (Dec.) 1969.*)

**PENTAZOCINE** Pentazocine produces subjective effects similar to those seen with morphine. However, at a dose of 60 mg/70 kg, pentazocine produces subjective effects which more closely resemble those of nalorphine than those of morphine. Pentazocine will not suppress abstinence symptoms in subjects dependent on either 60 or 240 mg/day of morphine. Pentazocine is 1/150 as potent as nalorphine in precipitating abstinence symptoms in subjects dependent on 240 mg of morphine per day. Long-term administration of pentazocine produces dependence which has elements of both morphine and nalorphine dependence. Pentazocine has an abuse potential which is less than that of morphine but greater than that of nalorphine. (*Jasinski, D. R., Martin, W. R., and Hocldtke, R. D.: Effects of Short- and Long-Term Administration of Pentazocine in Man, Clin. Pharmacol. Ther. 11: 385 (May) 1970.*)