ABNORMAL DEVELOPMENT IN *RANA PIPIENS* INDUCED BY COLCHICINE TREATMENT AT LATE GASTRULATION

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The narcotic alkaloid, colchicine, has been used extensively for a number of years in the experimental study of polyploidy in plants. It has been used to a less extent on animal tissue.

Among vertebrates, Waterman (1940) has found that colchicine has general retardative and inhibitory effects on the fish embryo, *Oryzias latipes*. This was indicated by developmental disturbances in the brain and eyes, the circulatory system, and in modifications of the tail.

Paff (1939) noted general retarded growth and anomalies of the nervous system of chick embryos after colchicine treatment. In addition, he produced evidence to show that colchicine may have an acceleratory effect as well as an inhibitory effect on mitosis.

Keppel and Dawson (1939) treated very early stages of *Rana pipiens* with colchicine in dilute concentrations over relatively long exposure periods. The results showed that colchicine induces a meroblastic type of cleavage and disturbs gastrulation. There was some indication of polyploidy. Rarely were embryos viable beyond the neural fold stage.

The eggs of *Rana pipiens* were also used in the present investigation. In contrast to the experiments of Keppel and Dawson, however, exposure to colchicine was at a later stage, at much higher concentrations, and for relatively short periods of time. Gastrulation was the stage chosen for treatment since the period up to, and perhaps through, this stage seems to be the most critical and because the effect of colchicine on organogenesis could be observed readily.

MATERIALS AND METHODS

The eggs for these experiments were obtained during the spring by injections of crushed pituitary glands and were fertilized artificially according to Rugh (1941).

All eggs were fertilized at room temperature, cut with dissecting scissors into groups of five, and then kept in finger bowls in a large, well insulated constant temperature cabinet at 18.0°C ± 0.3°C. The cabinet contained a light source that switched on and off automatically.

After fertilized eggs were placed in the constant temperature cabinet, development was allowed to continue normally for 42-43 hours (except one batch, series 14, in which development continued for 46 hours); i.e., embryos were permitted to develop until they had reached a stage in gastrulation between Shumway's (1940) stages 11 and 12 (mid- to late gastrula). Gastrulae then were placed in their respective colchicine concentrations (cooled to 18°C before use) in fresh greenhouse tank water. Control groups were placed in fresh greenhouse tank water. In general, embryos were fixed in Smith's fluid (Rugh, 1941) four days after treatment; *i. e.*, when the controls were at approximately Shumway stage 20 (hatching stage). Some of the embryos, however, were fixed in Smith's fluid twenty-four hours after treatment. Each series represents 140 eggs from a single female; 14 females were used. Treatment used is summarized in table 1.

All preserved series were divided into three groups on the basis of length of

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exposure to colchicine and the form of colchicine used (table 2); subdivisions for the various concentrations were retained in each group.

Puckett's dioxan technique (1937) was used in running up embryos for embedding in 56-58°C. paraffin. Embryos were sectioned at ten micra and stained with Delafield's hematoxylin and eosin.

RESULTS

A summary of the abnormalities and mortality obtained at the different colchicine concentrations for one- and four-hour exposure periods is given in table 2.

Observations of Living Material

For most of the series, colchicine treatment was begun about 4:00 P.M. By 7:30 A.M. of the next day, the retarding effect of colchicine was observable already in that all the embryos in the higher concentrations were still in the late gastrula stage. Usually after about twelve hours of colchicine treatment (longer when the crystalline form was used) a few scattered clumps of cells were observed in the dorsal lip region of some of the embryos in the higher concentrations. During the remaining part of the four days in which these embryos were allowed to develop,

TABLE 1

Treatment of Rana pipiens *at Late Gastrulation*

Series	Form of colchicine used	Colchicine concentrations	Exposure period	Fixation
1–6	Fresh amorphous powder	0.1%, 0.01%, 0.001% 0.0001%, 0.00001%, 0.000001%, plus control	1 hour	½ fixed 24 hours after treatment; ½ fixed 4 days after treatment.
7–10	Old crystals (kept in laboratory at least 2 years)*	0.4%, 0.2%, 0.1%, 0.05%, 0.01%, 0.001%, plus control	1 hour	Fixed 4 days after treatment
11–12	Fresh amorphous powder	0.4%, 0.2%, 0.1%, 0.05%, 0.1%, 0.001%, plus control	4 hours	Most fixed 4 days after treatment
13–14	Fresh amorphous powder	1.0%, 0.7%, 0.4%, 0.2%, 0.1%, 0.05%, plus control	1 hour	Most fixed 4 days after treatment

*Fresh powder unavailable for a short time.

these small light-colored clumps of necrotic cells increased in number, appearing to increase from the dorsal lip region forward. In the higher concentrations these sloughed off clumps of cells eventually formed a thick mass of cells along the neural groove. In these cases the closing of the neural folds was inhibited. Some of these clumps of necrotic cells appeared to be kept in constant motion within the inner jelly capsule by the ciliary movements of the embryo. These accumulations of sloughed-off cells were progressively less conspicuous in the lower concentrations.

Many of the experimental animals retained their yolk plugs much longer than did the controls. This was especially true of embryos in the higher concentrations, those in 1.0% colchicine retaining their yolk plugs the longest. In Series 14, 1.0% concentration, all animals fixed had persistent yolk plugs. A hatched embryo with a persistent yolk plug sometimes was observed moving around with yolk plug protruding; in others, exuding yolk was strewn around in the medium. This latter effect may be duplicated by stimulating the embryos with an instrument, after which muscular contraction "caused the yolk to exude," as Dawson (1938) aptly put it , "as a solid core resembling paste leaving a tube."

Most of the weakened embryos in the higher concentrations had failed to hatch

at fixing age, while only a few of the controls were unhatched when fixed. This was probably a natural result of the inhibitory effect of colchicine. Waterman (1940) also mentions the failure of weakened fish embryos to hatch after colchicine treatment.

Mortality of treated animals (table 2) was mainly in three batches, and apparently is correlated with concentration and length of exposure.

EXTERNAL OBSERVATIONS

The treated animals, in general, seem to fit into a graded series, the effect of the

TABLE 2

Abnormalities and Mortality of Treated Embryos
(Based on external observations)

	Conc. %	Total Embryos Treated	Mortality of Treated Embryos	Ratio Abnormals to Total Sample Treated	Abnormals %
	1.0	40	23	40/40	100.0
	0.7	40	22	40/40	100.0
Group I	0.4	39	22	39/39	100.0
	0.2	40	4 plus 1?	40/40	100.0
Series	0.1	94	1 plus 5?	94/94	100.0
1–6, 13,	0.05	40	1	22/40	55.0*
and 14	0.01	59	0	4/59	6.8
	0.001	60	0	1/60	1.7
Powder	0.0001	58	0	5/58	8.6*
1 hour	0.00001	59	0	3/59	5.1*
	0.000001	60	0	0/60	0.0
	Control	99	0	4/99	4.0*
Group II	0.4	79	2	79/79	100.0
	0.2	80	1	77/80	96.3*
Series	0.1	76	0	21/76	27.6*
7–10	0.05	79	0	1/79	1.3
	0.01	80	0	4/80	5.0*
Crystals	0.001	80	0	5/80	6.3*
1 hour	Control	78	0	6/78	7.7*
Group III	0.4	40	30 plus 7?	40/40	100.0
	0.2	40	18 plus 3?	40/40	100.0
Series	0.1	40	11 plus 7?	40/40	100.0
11 and 12	0.05	40	5 plus 2?	40/40	100.0
	0.01	39	0	0/39	0.0
Powder	0.001	40	0	0/40	0.0
4 hours	Control	39	0	2/39	5.1*
Total	Controls	216	0	12/216	5.6*

‡Embryos treated minus those killed accidentally as a result of handling.
*Many of the embryos represented by these percentages were only slightly abnormal in appearance.

colchicine treatment increasing with increasing concentrations. There is a general inhibitory effect resulting in stunted growth. One of the most marked effects is a gradual reduction in head size until the most antero-dorsal part of the embryo is on a line with the suckers instead of protruding beyond them as in the controls. Varying degrees of microcephaly have also been noted by Hoadley (1938) when embryos were kept at supramaximal temperatures, and by Briggs (1941) when embryos were permitted to develop following delayed fertilization. Other marked effects noted in the present investigation are total inhibition, partial retardation,

TABLE 3

*Summary of Histological Observations**

	COLCHICINE CONCENTRATIONS						
	1.0%	0.7%	0.4%	0.2%	0.1%	0.05%	0.01–0.00001% and control
Suckers	Almost normal development	Almost normal development	Normal	Normal	Normal	Normal	Normal
Olfactory pits	Absent	Absent	Absent	Absent	Absent	Absent	Normal
Eyes	Absent	Absent	Absent	Absent	Optic stalk and optic vesicles present; no lens	Optic stalk and cup show infiltration; lens present	Normal: optic stalk, vesicles, and lens
Otic vesicles	Absent	Absent	Absent	Absent	Differentiated but retarded	Normally differentiated; abnormal in position	Normal
Brain	Absent	Absent	Absent	Solid cord of cells surrounded by cavity	Differentiated but retarded	Larger than in 0.1% but still retarded	Normal: cavities free of infiltrated cells
Nerve Cord	Solid mass of indifferent cells	Solid mass of indifferent cells	Solid, some cellular differentiation	Solid and differentiated	Tubular; contains infiltrated cells	Tubular; normal differentiation; heavy infiltration	Normal: tubular, and canal free of cells
Hypophysis	Absent	Absent	Absent	Absent	Anlage present	Normal?	Normal
External gills	Absent	Absent	Absent	Absent	Anlage present	Arteries present; retarded	Normal, containing afferent arteries
Thyroid	Absent	Absent	Absent	Absent	Anlage present	Normal	Normal
Gut	Blocked with yolk; undifferentiated lining	Blocked with yolk; undifferentiated lining	Blocked with yolk; lining regular in outline	Mid-gut and pharynx differentiated (infiltrated)	Differentiated and free of foreign cells	Normal	Normal
Liver	Absent	Absent	Absent	Absent	Present?	Present?	Normal
Notochord	Filled with cells containing yolk and pigment granules	Filled with cells containing yolk and pigment granules	Fewer yolk-containing cells	Yolk present	Almost free of yolk	Normal	Normal: vacuolated and free of foreign cells
Heart	Absent	Absent	Absent	Absent?	Primordia?	Atrium and ventricle present; abnormally small	Normal: atrium and ventricle
Pericardial cavity	Absent	Absent	Large somewhat irregular cavity	Present; dorsal mesocardium connected to yolk	Abnormally large	Abnormally large; ventro-posterior yolk mass	Normal
Somites	Undifferentiated mass	Undifferentiated mass	Slightly differentiated	Indistinct	Incompletely differentiated	Differentiated	Normal
Coelom	Absent	Absent	Indicated by right and left cavities	Irregularly formed	Differentiated	Normal?	Normal
Pronephric tubules	Absent	Absent	Absent	One tubule (left)	Present	Normal?	Normal

**Rana pipiens* embryos treated with colchicine solutions of given concentrations for one hour, compared with untreated (normal) embryos (Shumway stage 20–21); incubation period, 140 hours; temperature, 18° C.

or malformation of tails; an increasing failure to close the blastopore; mild oedema; reduction or complete inhibition of external gills; and a dorsal flexion of the tail giving a "swayback" appearance to embryos in the intermediate concentrations. Waterman (1940) noted a number of these effects on fish embryos.

Effects which do not seem to be progressive but rather occur at random throughout the concentrations producing abnormals are the following: (1) marked oedema giving the embryos a bloated appearance, and (2) occurrence of smooth-surfaced, ectodermal, irregularly placed enlargements, the latter noted mainly in the dorsal region. The latter appear to be similar to the epidermal "blisters" noted by Mills (1939) after treating hatching tadpoles with colchicine. In the present experiments, an occasional extremely bloated embryo was permitted to develop beyond the four-day period that was allotted the others after colchicine treatment. Inflation increased in these animals until they died four or five days later.

It was found desirable to separate the series treated with fresh amorphous powder from those treated with the somewhat aged crystals (used for a short time when fresh powder was unavailable), and those treated for four hours from those treated for one hour. The powder used appeared to be more toxic than the crystals and the four-hour treatment more effective than the one-hour treatment (table 2).

Histological Observations

Histological studies are based on serial sections chiefly of the embryos of group I, one-hour powder treatment. These results are summarized in table 3. Because of apparent batch susceptibility, care was taken to section examples of the same batch wherever feasible. The embryos treated with a 1.0% colchicine solution and those treated with a 0.7% solution are very similar. The extreme retardation of these embryos which have persistent blastopores may be noted in table 3. The ventral yolk mass is reduced caudal to the head region, as indicated by an irregular cavity containing scattered clumps of yolk. Yolk is found in all the internal structures mentioned, as are scattered pigment granules. What appear to be abnormally large nuclei are present in the notochord and somites.

An embryo treated with a 0.4% concentration is, in gross appearance, similar to those in the two higher concentrations. Internally, however, it is not markedly inhibited (table 3). The pericardial cavity appears as a large, somewhat irregular space, divided dorso-ventrally into two parts by a mass of cells containing yolk. Small coelomic cavities are indicated by two spaces (right and left) located laterally and ventral to the notochord. The spinal cord region shows slight differentiation as compared with the control. The notochord contains fewer cells infiltrated with yolk granules so that it appears more vacuolated. The somites show some differentiation, and the gut lining is regular in outline, though the gut cavity still is blocked with yolk.

In a sectioned embryo which had been treated with the 0.2% solution (table 3), a solid cord of cells enclosed by a cavity appears in the prospective brain region. The nerve cord is still solid but differentiated. The mid-gut has a differentiated cellular lining. Indistinct somites are present, as is an irregularly formed coelom and a left pronephric tubule. The pharynx is differentiated but contains a few cells infiltrated with yolk. The pericardial cavity and what appears to be the dorsal mesocardium are present, but the latter is connected ventrally to a mass of yolk lying in the floor of the pericardial cavity.

The sections of an embryo treated with a 0.1% concentration (table 3) show a differentiated but retarded pharynx and brain. The primordia of the thyroid and hypophysis are present. The optic stalks and optic vesicles are present but the lens did not form. Otic vesicles containing a few cells infiltrated with yolk differentiated but are retarded in development. A nerve cord differentiated with a neurocoel but the latter contains a loose mass of cells. External gill primordia are present as evidenced by a slight protrusion on both sides of the head. Also

present are an abnormally large pericardial cavity, incompletely differentiated somites, a gut cavity free of foreign cells, pronephric tubules, a differentiated coelom and a vacuolated notochord practically free of cells containing yolk. The pericardial cavity merges caudally into an irregular cavity beneath the gut. The sections show a loose mass of infiltrated cells dorsal to the neural tube, caudal to the pericardial region.

An embryo treated with 0.05% concentration of colchicine has all the structures indicated in table 3 except the olfactory pits. The brain is larger than in the 0.1% concentration but still retarded, and the otic vesicles are normal as to differentiation but abnormally posterior in position. The external gills contain afferent branchial arteries but are somewhat retarded in development as compared with the control. The pericardial cavity is abnormally large. The tubular nerve cord is differentiated but is infiltrated heavily with cells, as are the cavities of the optic stalk and the optic cup. The heart is abnormally small and a mass of yolk is present in the posterior portion of the floor of the pericardial cavity.

All sectioned embryos of group I treated with more dilute concentrations than those described above appear normal.

Embryos of group III that were sectioned serially indicate, as did those of group I and group II, that increasing the concentration increased the toxicity of the drug. Sections demonstrate, however, that the toxic effects in group II (crystalline form) are not as great as those in group I where powdered colchicine was used; and the toxic effects in group I (one-hour treatment) are not as great as those in group III (four-hour treatment) for any given concentration.

DISCUSSION

The present investigation deals with the effects of colchicine on one stage of development in the frog, namely, the yolk plug stage. Since this research was done, Colombo (1944, 1947) and Samartino and Rugh (1946) have reported the effects of colchicine on various stages in frog development. The concentrations that these authors used, however, did not include concentrations as high as the present writer used. Colombo used longer exposure periods (ranging from one to twenty-five days) than those used in the present investigation. Samartino and Rugh did not indicate the length of exposure to colchicine. The present paper includes the effects of relatively high concentrations (up to 1.0%) acting for short exposure periods (one hour and four hours). The results demonstrate that gastrulae of *Rana pipiens* are able to tolerate colchicine concentrations up to 0.01% for short periods without teratological effects, and may live several days following exposure to chochicine. At 0.05% concentration, the four-hour powder treatment was more effective than the one-hour powder treatment in producing abnormalities.

The toxicity of colchicine has been shown to be correlated to some degree with temperature. Fühner, and Fühner and Wagner (Fühner and Breipohl, 1933) observed that colchicine is far more toxic at warm temperatures than at cold. The present work tends to confirm this observation since in preliminary experiments a high percentage of abnormalities was obtained in concentrations of 0.01% and 0.001% when yolk plug stages were treated for one hour at room temperature (22-24°C), while at a temperature of 18°C no significant percentage of abnormalities was obtained. It was, therefore, necessary to resort to higher concentrations in order to obtain a graded series of abnormalities at 18°C, a temperature convenient for comparison with Shumway's stages for the development of *Rana pipiens* (1940). Colombo (1947) states that *Rana agilis* appears to be much less susceptible to the action of colchicine than is *Rana esculenta* which he used in his first series of experiments (1944). He attributes this, in part, to a difference in rate of development between the two species and, in part. to the difference in temperature at which the two series of experiments were run (*Rana agilis* at 7-11°C and *Rana esculenta* at 17-23°C). He states that the diffusibility of colchicine into the cytoplasm would probably be faster at higher temperatures and vice versa. His results, therefore,

tend to confirm the observations presented here. It should be pointed out, however, that raising the temperature may induce teratological effects (Hoadley, 1938) by abnormally increasing the rate of development and thus may be producing an indirect effect.

The most marked effect of colchicine noted on living embryos, besides general retardation, was the sloughing of cells beginning around the dorsal lip and later extending more anteriorly in the region of the neural groove. The ciliary motion of the developing embryos probably freed these clumps and then set them moving in every direction. As more and more of these clumps of cells became free and finally congregated along the wall of the inner capsule, it was difficult to determine whether an embryo was alive unless it was actually observed moving within its capsule. This effect was due apparently to the death of the sloughed cells which congregated along the inner wall, causing the capsule to appear whitish and opalescent. Paff (1939) *et al* noted a sloughing of the ectoderm in the chick embryo after colchicine treatment. Solberg (1938) noted the extrusion of injured cells from tissues following x-ray treatment. The fact that the sloughing began at the dorsal lip is in accord with his observations that during gastrulation the dorsal lip and the invaginating tissue are the most sensitive parts of the fish embryo.

The persistent yolk plugs obtained after colchicine treatment have also been noted after dinitrophenol treatment, of *Rana pipiens* (Dawson, 1938) and of fish embryos (Waterman, 1939). Persistent yolk plugs and delayed closure of the blastopore also have been obtained by keeping developing eggs at subminimal and supramaximal temperatures (Hoadley, 1938; Atlas, 1935). Hoadley (1938) and Dawson (1938) mention loss of yolk and atypical closure of the blastopore. Waterman (1940) mentions the failure of weakened embryos to hatch. Thus the morphological effect of colchicine is apparently a non-specific one.

On the basis of external observations, the treated animals, in general, seem to fit into a graded series, the effect of the colchicine increasing with increasing concentrations. Colombo (1944) also noted this after colchicine treatment of the yolk plug stage of *Rana esculenta* and *Bufo vulgaris*, and Waterman (1940) noted it after colchicine treatment of fish embryos. It should be pointed out, however, that differential susceptibility exists among the series. This differential batch susceptibility is noted especially in the 0.1% concentration of group I in the present investigation. This is the concentration having the greatest number of series represented, and hence the greatest number of individuals. The series within this single concentration can be put into several different groups according to their degree of retardation. There is also individual susceptibilty within series. What seem to be apparent differences in susceptibility, however, may be due, in part, to the impossibility of selecting identical stages, as Dawson (1938) suggests.

The apparent difference in toxicity between the powder and the crystals may be due to either the form of the colchicine used or the age of the form used, the amorphous powder having been fresh and the crystals having been kept in the laboratory for at least two years. The latter cause is more probable since it is known that colchicine deteriorates with age—at least, if care is not taken to keep it in complete darkness (U. S. Pharmacopoeia, 1936).

It has been pointed out that the four-hour treatment was more effective in producing abnormalities than the one-hour treatment. Colombo (1947) also noted that the toxic effect of colchicine is proportional to the duration of its action when frog embryos are treated at the blastula stage or later, and Waterman (1940) has noted this effect on fish embryos.

Some of the externally visible abnormalties that have been described in the present paper also have been reported by Wolsky (see Colombo, 1944) and by Colombo (1944, 1947) after treatment of gastrulae and other stages with colchicine. These abnormalities include general retardation, microcephaly, acephaly, extrusion of yolk, and oedema (indicated by swelling of the ventral region). Macroscopic abnormalities described by Samartino and Rugh (1946) include meroblastic cleav-

age, exogastrulation, microcephaly, acephaly, stunting, distortion, wrinkling of body ectoderm, curvature of the body.

Many of the types of abnormalities produced in frog embryos by colchicine are similar to those produced by other environmental, and also by internal, factors. A number of these similarities has been noted earlier in this paper. The smooth-surfaced protrusions from the body wall is one type of abnormality described by Briggs (1941) following delayed fertilization. Oedema sometimes, at least, is associated with haploidy and osmotic imbalance (Rugh, 1941). The latter was noted by Piiper (1933) after treatment of frog embryos with cane sugar solutions, and by Baldwin (1919) and Rugh (1950) after x-irradiation of frog embryos. The "swayback" embryo and other types of abnormalities illustrated by Rugh (1941, abnormalities 80 and 84) as being found frequently in parthenogenetic or androgenetic tadpoles were noted after colchicine treatment in the present experiment.

A study of serial sections revealed that large irregular ventral spaces are often present in the yolk mass of those embryos succumbing to the toxic effects of colchicine. Probably these spaces are due to a progressive loss of yolk, as suggested by protruding yolk plugs and by exuding yolk in living embryos. A study of sections suggests that the infiltration of cells noted in the neurocoel and accessory cavities (table 3) represents the sloughed cells noted in the living material. These cells appear to have been caught within the neurocoel when the neural folds were closing to form the neural tube. Masses of cells in the neural canal have been noted after experimental procedures involving dinitrophenol (Waterman, 1939; Dawson, 1938) and after x-ray treatment (Solberg, 1938; Baldwin, 1919). In higher colchicine concentrations, the nerve cord is represented only by a mass of incompletely differentiated cells in which the neurocoel is absent. These conditions are similar to those found in embryos that have developed from over-ripe eggs (Witschi, 1930). In the same paper, Witschi described an excess of brown pigment granules in treated eggs (compare table 3 of present paper).

Table 3 indicates also that cells containing yolk were found in the notochord, giving the normally vacuolated notochord a more or less solid appearance in the higher concentrations. This also has been described by Colombo (1944), and a similar situation has been described in frog development after treatment with cane sugar solutions (Piiper, 1933). This latter paper also describes the gut as being partially blocked with yolk, as was noted in the present experiment, and by Hoadley (1938).

Failure of the heart to differentiate (see table 3) also has been noted by Solberg (1938) and by Baldwin (1919) after x-ray treatment; irregularities in heart form were mentioned by Waterman (1940) after colchicine treatment of fish embryos.

Histological results, in general, seem to indicate that organs are retarded progressively (or completely inhibited) as embryos are exposed to progressively higher colchicine concentrations, depending to a certain extent on their position along the embryonic axis. In the present investigation, the brain is the most markedly affected part of the central nervous system. The sense organs are inhibited progressively: the olfactory pit is absent in the least effective concentration used (table 3); the optic vesicles are inhibited in the next higher effective concentration; and the otic vesicles are inhibited markedly only in still greater concentrations. In the digestive system the pharynx is inhibited by concentrations in which the mid-gut is differentiated. Colombo (1944) notes that the differentiation of the pharynx and gills is related to the normality of the brain, their organization being poor if the brain is abnormal. This relationship is suggested in table 3, especially in the case of the gills. Solberg (1938), Colombo (1944), and Samartino and Rugh (1946) have pointed out that there is a fundamental difference between cells dividing without differentiation and those about to undergo differentiation. This is shown by their differential susceptibility to colchicine, to other chemical agents, and to x-ray treatment. Some of the effects of these external factors have been described in the present paper.

The exact mechanism by which colchine acts is unknown; as Colombo (1944) points out it does not produce a direct toxic effect but acts through individual cells. It is well known that colchicine acts on both plant and animal cells by inhibiting mitosis, which may result in polyploidy (Bergner, Avery, and Blakeslee, 1940; Allen and Creadick, 1937; *et al.*). Its action of preventing spindle formation by preventing the rise in viscosity which normally accompanies cell division has been demonstrated (Wilbur, 1940; Beams and Evans, 1940; *et al.*). The primary effect of colchicine, then, is on cellular action. Colombo (1947) points out that during cleavage and gastrulation colchicine acts primarily upon nuclei. At later stages he concludes that colchicine affects the utilization of yolk and interferes with water metabolism in the developing embryo. The former is suggested in the present investigation in which yolk granules have been seen dispersed throughout most of the organ primordia at the higher concentrations of colchicine. Also in support of this, Keppel and Dawson (1939) have described a progressive vacuolation in the uncleaved yolk mass of abnormal blastulae following treatment with colchicine. In some little understood way colchicine also seems to interfere with water metabolism not only for the single cell but for the embryo as a whole. Colombo (1944) points out that the pressure of imbibition produced in embryos treated with colchicine would impede gastrulation and by increasing the total water intake would produce oedema and enlargement of the pericardial cavity. Similar results have been obtained in the present investigation.

SUMMARY

The eggs of *Rana pipiens* were obtained by pituitary injections, separated into small groups, and a short time after fertilization incubated at 18°C. They were allowed to develop at this temperature until fixed, except for brief periods during which they were removed to room temperature for observations. Embryos were treated with colchicine when they were between Shumway stages 11 and 12 (late gastrula). They were fixed at Shumway stage 20 (hatching stage).

The results indicate that, within the limits of the experiments, colchicine activity is correlated with—

(1) temperature: at higher temperatures (22°-24°C in preliminary experiments) colchicine is more toxic than at lower temperatures (18°C);

(2) length of exposure: the longer the exposure to the drug, the greater the effect;

(3) concentration: the higher the concentration used, the greater the effect;

(4) either the form of colchicine used or the age of the drug used: either the amorphous powder is more toxic than are the crystals, or the length of time the crystals were kept before being used reduced their potency. The latter is more probable.

Mortality after colchicine treatment is correlated with concentration and length of exposure.

Some of the readily observable external effects of colchicine on frog embryos are general retardation and inhibition involving a disturbance of the cephalocaudad axis, microcephaly, oedema, persistent yolk plugs, and ectodermal enlargements.

Histological observations indicate that the degree of sensitivity of the organs studied is correlated with their degree of differentiation and to a certain extent with the position of the developing organs along the embryonic axis. There was some indication of polyploidy in that many of the nuclei appeared abnormally large.

It was noted that the abnormal effects produced by colchicine in *Rana pipiens* are similar to abnormal effects induced by other treatments, such as the action of dinitrophenol, cane sugar, x-ray, extremes of temperature, and delayed fertilization. It, therefore, cannot be said that any of the effects noted are specific for colchicine.

The exact mechanism by which colchicine acts is unknown. Its primary effect is a cellular one, for during cleavage and gastrulation it seems to act primarily on nuclei. At later stages colchicine disturbs the utilization of yolk and interferes with water metabolism in the developing embryo.

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