adjusted to approximately 7 with concentrated hydrochloric acid.

The approximate oral LD_{50}, expressed as mg per kg of body weight, for myosmine and *l*-nicotine was 1875 and 188 respectively. After intraperitoneal administration the approximate LD_{50} for myosmine and *l*-nicotine was 190 and 30 respectively. The symptoms of toxicity produced by the 2 alkaloids were essentially the same, although the convulsions produced by large doses of myosmine were somewhat less violent than those produced by *l*-nicotine.

Segments of either intestine or uterus were suspended in a 50 cc bath of Ringer-Locke solution at 38°C and connected to a frontal writing lever for kymographic recording. In each case the effect of *l*-nicotine was first determined before myosmine was added.

Myosmine in concentration of 4 mg/50 ml (1:12,500) caused contraction of the isolated intestinal segments similar to that produced by *l*-nicotine in concentrations of 0.02 mg/50 ml (1:2,500,000). On the isolated uterus no effect was produced by either myosmine or *l*-nicotine.

Recently Pick, Brooks and Unna[1] have reported on the inhibitory effect of sulfathiazole on the activity of *l*-nicotine on the intestine. We have confirmed their studies and have extended this procedure to include myosmine.

Like nicotine, the effect of myosmine was inhibited by suitable doses of sulfathiazole. Removal of the sulfathiazole from the bath and washing the segments with fresh Ringer-Locke solution promptly restored the sensitivity of the intestinal segment to both myosmine and *l*-nicotine. The results are illustrated in Fig. 1-3. A detailed description of the individual tracings is given under the figures.

Summary. 1. The acute toxicity of myosmine was about one-tenth that of *l*-nicotine after oral administration. Intraperitoneally myosmine was about one-sixth as toxic as *l*-nicotine. 2. On isolated intestinal strips of guinea pigs, contraction by myosmine required about 200 times the concentration necessary in the case of *l*-nicotine. 3. Sulfathiazole inhibited the action of both myosmine and *l*-nicotine on isolated intestinal strips.

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Effects of Colchicine on the Frog in Relation to Ovulation and Early Development.

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The property of inhibiting mitosis and inducing polyploidy[1-3] has made of colchicine a valuable tool in biological research. It has been used in studies on the rate of mitosis in various tissues.[4-6] It has been demonstrated that colchicine prevents the rise in viscosity[7-9] in the cell which normally occurs at fertilization and cell division and also lowers the surface tension in the dividing cell.[10] It has been used in studies on malig-

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[2] Kostoff, O., *Nature*, 1938, **142**, 753.

[3] Maba, F., *Jap. J. Genetics*, 1939, **15**, 344.

[4] Brues, A. M., *J. Physiol.*, 1936, **86**, 63.

[5] Allen, E., and Credick, R. N., *Anat. Rec.*, 1937, **69**, 191.

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[7] Beams, H. W., and Evans, T. C., *Biol. Bull.*, 1939, **77**, 328; 1940, **79**, 188.

[8] Nebel, B. R., and Ruttle, M. L., *J. Heredity*, 1938, **29**, 3.

[9] Wilbur, K. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 696.

[10] Wada, B., *Cytologia*, 1940, **11**, 96.

nant cells.[11-14] The series of experiments herein reported were done with the purpose of seeking explanations for various phenomena that occur during amphibian ovulation, fertilization and early development.

Materials and Methods. The experiments were of 2 kinds: *in vivo* and *in vitro*. In the *in vivo* experiments, adult, preovulatory female and adult male *Rana pipiens* were injected in the abdominal cavity for a number of days with colchicine in solution. After several days the females were injected with an excessive dose of pituitary glands in order to induce them to ovulate, and the eggs fertilized with a sperm suspension of minced testes.[15] In the experimental males the left testis was fixed for histological examination and the right was used to fertilize normal eggs. The motility and virility of spermatozoa, as used in suspensions for fertilization, were also tested. The *in vitro* experiments consisted of exposing eggs of *Rana pipiens,* at various stages of development, to varied concentrations of colchicine at different temperatures, but keeping a constant volume of 2 ml of solution per egg or larva.

Experimental Results. The physiological and toxic reactions of the adult frogs to the drug were in accordance with what is known.[16] A pink fluid was found in the subcutaneous lymph spaces. There was a diffuse congestion and inflammation of the mucous membranes of the abdominal viscera. There were numerous small petechiae on the surfaces of the muscles of the limbs, under the skin. There was a blanching of the kidney and adrenal. The gastro-intestinal mucosa was markedly inflamed and the intestines were distended with gas and a frothy mucus. The animals became sluggish, lost the corneal reflex, their proprioceptive senses, and showed a lack of limb coordination. With certain doses of colchicine (varying greatly in different frogs: 0.05 mg, 0.1 mg, 0.2 mg, 0.5 mg, 1.0 mg, and 2.0 mg O.D.) ovulation could be suppressed. In some cases a few eggs were shed (less than a hundred), but these could not be fertilized by the usual technic.[15] In the injected males there was no effect upon the sperm, nor did the histological picture of the testes change with a dosage of 0.05 mg O.D. However, *in vitro* it was found that sperm in concentrations of colchicine from 1:1.000 to 1:70,000 retained their motility (for at least 1½ hours), but lost their ability to activate the eggs. None of the eggs so exposed to spermatozoa ever developed, though most of them rotated.

In the *in vitro* experiments it was found that the developing egg could not survive stages No. 7, No. 12, No. 14-15 in the more concentrated solutions of colchicine (1:10,000 and stronger); eggs in weaker solutions showed meroblastic cleavage. Eggs in colchicine solutions of 1:50,000 and weaker (up to 1:1,000,000) showed little or no deleterious effects. Eggs placed in colchicine after stage No. 7 continued to develop but died at stage No. 12 as exogastrulae. Eggs placed in colchicine after stage No. 12 went on to stage No. 14-15 where the neural folds never fused, and the larvae showed tendencies toward acephalia and microcephaly. Eggs placed in colchicine after stage No. 16 never seemed to be able to hatch out of the jelly capsule, yet if the capsule were ruptured for them, they went on to develop apparently normally. In cases where the larvae were subjected to colchicine just as an organ anlage was about to start to grow, the anlage was suppressed, and even after the larva was removed from the solution and returned to spring water, the anlage failed to develop, *e.g.* the gill did not develop at all or else it grew markedly stunted and without circulation.

In cases where embryos survived the exposure to the colchicine solution and developed through stages No. 7, No. 12, No. 14 they showed a tendency toward microcephaly,

[11] Dustin, A. P., *Bull. Acad. Roy. Med. Belg.*, 1934, **4**, 487.

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[13] Amoroso, E. C., *Nature*, 1935, **135**, 266.

[14] Clearkin, P. A., *J. Path. and Bact.*, 1937, **44**, 469.

[15] Rugh, R., *Experimental Embryology*, New York University Bookstore, 1942, p. 25.

[16] Cushny, A. R., *Pharmacology and Therapeutics*, Lea and Febiger, 1940, p. 686.

and a few appeared to be acephalous. In addition these embryos showed stunting, distortion, wrinkling of the ectoderm, curvatures of the body, poorly developed gills without circulation, and exogastrulation. In all the cases the rate of development of the experimental larva lagged behind that of the control; the greater the concentration of colchicine, the greater the lag. In no instance was the size of the experimental greater than that of its control.

Microscopic examination failed to show any differences in the testes; it showed the involution in meroblastic cleavage to have been normal in mechanism, but that it had occurred at a much higher point of latitude on the egg mass than normally, leaving a large amount of yolk unincorporated, so much so that often hemi-embryos resulted which soon died. Mitotic (metaphase) figures were scarce. However, there was an abundance of apparently resting and pycnotic nuclei. In *Rana pipiens* it was noticed that some of these nuclei were 2 and 3 times the size of a normal nucleus, either in discrete masses or bound together by thin filaments of chromatin material.

Discussion. The presence of the pink fluid in the subcutaneous lymph spaces and the blanching of the kidney and suprarenal body, indicate either a loss of red blood cells with subsequent hemolysis, or hemolysis within the blood vessel and subsequent excretion of free hemoglobin by the kidney, and loss intercellularly in the capillary beds of the lymph spaces. The evidence that colchicine is a capillary poison is evidenced by the marked dilatation of the capillaries. The great inflammation present in the intestines can be accounted for, since this is the chief site of the excretion of the drug. The insensitivity of the animals to mechanical stimuli is probably the result of paralysis of nerve endings, and the depression of sensory and motor centers in the central nervous system.[17]

The mechanisms by which colchicine suppressed ovulation, and destroyed the virility of the sperm but not their motility, are moot questions. Clearly, rotation of the egg cannot be accepted as a criterion for penetration by the sperm, for it seems improbable that of all the eggs observed to rotate (several thousand), not one should have cleaved, even on a pathenogenetic basis.

When one considers that the gross deformities in the embryos resulted from a lack of cell division in some areas, and from unequal or unbalanced growth, it becomes evident that there is a qualitative difference in cells about to undergo differentiation and those that are dividing without differentiation. This is shown by their differential susceptibility not only to colchicine, but also to other chemical agents as well.[18,19]

Opinion is divided on the question of whether colchicine is a mitotic stimulant or inhibitor.[20–22] From our experiments we must conclude that it acts as a depressant in mitosis in the frog. The drug prolongs the mitotic cycle, and in certain concentrations stops it completely. The mechanism can only be speculated upon. Yet, the experimental evidences of many workers tend to support Bernal's theory[23] of negative tactoids in mitosis and spindle formation. The lack of the requisite increases in viscosity, due to the influence of colchicine, may account for the lack of spindle formation[7,24–26] and for the bizarre figures seen in colchicine-treated cells.[20]

It has been stated[27] that, as a result of their reactions to colchicine, anuran and urodelan tissues do not constitute a homogene-

[17] Jacobi, K., *Arch. f. exp. Path. und Pharm.*, 1890, **27**, 119.

[18] Waterman, A. T., *Biol. Bull.*, 1939, **76**, 162; 1940, **78**, 29.

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[23] Bernal, J. D., *Cell and Protoplasm*, 1940, p. 199.

[24] Brues, A. M., and Jackson, E. B., *Am. J. Cancer*, 1937, **30**, 504.

[25] Ludford, R. J., *Arch. f. exp. Zellforsch.*, 1936, **185**, 411.

[26] Barber, H. N., and Callan, H. G., *Proc. Roy. Soc. (B)*, 1943, **131**, 258.

[27] Mills, K. O., *J. Morph.*, 1939, **64**, 89.

ous class of tissues. Incidental observations on *Amblystoma punctatum* demonstrated that the number of pycnotic nuclei and metaphase figures were not the same in the frog and salamander. Yet, if one considers the pycnotic nuclei as former metaphases which have become agglutinated, and the pseudo resting stages as transitionals from the colchicine metaphase figure to the pycnotic nucleus, then the sum total of all 3, in each species, is about equal. Then, the reaction to colchicine is qualitatively and quantitatively the same in both the frog and the salamander, both microscopically and grossly.

Summary and Conclusions. Colchicine was used in concentrations ranging from 1:1000 to 1:1,000,000. It was found to act as a capillary poison, to prevent ovulation when injected into sexually-mature female frogs prior to anterior pituitary stimulation, to prevent cleavage of normal eggs exposed to spermatozoa previously treated with colchicine, to affect gastrulation to such an extent that exo-gastrulae were the rule, to inhibit the appearance of organ anlage when applied just before the normal appearance time, to retard general growth of the larvae to such an extent that cephalic structures were markedly stunted and hatching prevented. The cytological picture was one of many pycnotic nuclei, occasional multinucleated cells, few metaphase figures, and apparent resting stages of mitosis.

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Simultaneous Cultivation of Typhus Rickettsiae and Influenza Virus in the Developing Chick Embryo.

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Over the past few years, large numbers of developing chick embryos have been used in this laboratory for the production of vaccines prepared from typhus fever rickettsiae and from influenza virus. In the preparation of typhus vaccine, the rickettsiae are cultivated in, and harvested from the yolk sac, whereas for influenza vaccine, the influenza virus is harvested from the allantoic fluid. In each instance, the remainder of the egg-contents is discarded. Thus, the allantoic fluid of the typhus eggs and the yolk sacs of the influenza eggs are wasted in the preparation of typhus vaccine and influenza vaccine, respectively. It had been observed, however, that in eggs inoculated with typhus rickettsiae in dilutions that did not result in the early death of the embryo, very few rickettsiae were found in the allantoic membranes. Moreover, it did not seem likely that rickettsiae, even if present, would be adsorbed on and subsequently eluted from fowl red blood cells, after the manner of the influenza virus.[1] Conversely, the adapted form of influenza virus used as seed material for vaccine production does not proliferate to any extent in the yolk sac.[2] In each instance, then, the portion of the egg that is discarded could be salvaged and used to advantage if both agents could be grown simultaneously in the same egg. The present communication records the results of a series of experiments in which vaccines were prepared from eggs inoculated with both typhus rickettsiae and influenza virus.

In preliminary experiments, it was found that an egg previously infected with typhus could actually be reinfected with influenza. In order to obtain a good yield of influenza, it would be essential, however, to regulate

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[2] Beveridge, W. I. B., Burnet, F. M., and Williams, S. E., *Australian J. Exp. Biol. and Med. Sc.*, 1944, **22**, 1.