15417

Abnormalities of Amphibian Development Following Exposure of Sperm to Colchicine.*

THOMAS S. HALL. (Introduced by Wm. A. Hiestand).

From the Department of Biology, Purdue University, West Lafayette, Ind.

The familiar effects of colchicine alkaloid upon mitosis point to the possible desirability of exposing male and female sex cells to this substance separately. Results of such experiments have recently been reported, for Drosophila, by Hadorn and Niggli.[1] For the grass frog (*Rana pipiens*) they are reported herewith.

In a preliminary experiment sperm were exposed to a commercially prepared emulsion which contained colchicine and a number of other substances. A majority of eggs fertilized by these sperm developed abnormally. This result raised 2 questions: (1) What substance (or substances) in this preparation was responsible for the abnormalities observed? (2) What was its mode of action?

In order to answer the first question, approximately one-half of each testis of a pithed healthy male frog was macerated in tap water containing 0.04% of chemically pure colchicine in solution. The remaining portions of the 2 testes were macerated in tap water without colchicine. Into each of these suspensions, 3 hours later, about 600 eggs were introduced. These were obtained from 2 females induced to ovulate by the method developed by Rugh.[2]

The general result was as follows. Cleavage occurred in about 97% of eggs fertilized by non-treated sperm as compared to 72% fertilized by treated ones. Of the latter group, a very few (about 8%) developed normally; all the rest abnormally. The types of abnormality were apparently not different from those secured by Keppel and Dawson[3] who exposed whole fertilized eggs to colchicine and described the resulting anomalies in detail. The effects also considerably resemble those which many workers have produced by abnormal heat and pressure, by many different organic and inorganic chemicals, and by various surgical procedures. The literature describing such experiments generally reveals 2 frequently recurring results: (1) a single chemical agent can produce many different kinds of abnormality; (2) different numbers of the various types appear in different experiments even when the same treatment is applied. The results of exposing sperm to colchicine did not differ in these respects. Table I gives the numbers of different effects in a representative sample of 100 individuals chosen at random.

A special study was made of cases of partial cleavage. Usually most of the darkly pigmented portion of the animal half cleaved. In some instances, however, less than half of the animal region was involved, and this in a few cases included part of the gray crescent. Since the noncleaving portion might unfavorably affect the cleaving portion, the latter, in 20 cases, was carefully detached from the former by means of the Spemann hair loop. Twelve of these were neuroectodermal fragments. They had been underlain by no invaginated material prior to removal. They lived 3 to 5 days after removal, undergoing no further differentiation. The other fragments included presumptive mesoderm. They lived 4 to 6 days, undergoing both differentiation and regulation into extremely abnormal, histologically complex masses of tissue. In their developmental behavior, these fragments resembled those which have previously been isolated from the corresponding regions of normal individuals and have been studied

* The main substance of this paper was reported at a meeting of the Purdue Biological Society, March 17, 1946.

1 Hadorn, E., and Niggli, H., *Nature*, 1946, **157**, 162.

2 Rugh, R., *Biol. Bull.*, 1934, **66**, 22.

3 Keppel, D. M., and Dawson, A. B., *Biol. Bull.*, 1939, **76**, 153.

TABLE I.
The Number of Individuals Manifesting Each Type of Abnormality Is Indicated in Parentheses.

Cleavage	Gastrulation	Neurulation	Age in days at death	Condition at death
Cleavage fails to occur (28)	Gastrulation fails to occur (28)	Neurulation fails to occur (28)	?	Radical, nonuniform redistribution of pigment
Cleavage occurs in a restricted region (18)	See discussion in text (18)	See discussion in text (18)	3-6	See discussion in text
Total cleavage occurs (54)	Not all of yolk enclosed (40)	Neurulation fails to occur (28)	4-5	Incompleted gastrulae; yolk often vacuolated
		Neural tubes small and deformed (12)	4-16	Small monstrous embryos with much yolk exposed; yolk often vacuolated
	Blastopore closes after neurulation begins (10)	Neural tubes small; closing delayed at posterior end (10)	6-16	Microcephalic monsters; two with spina bifida
	Gastrulation apparently normal (4)	Neurulation apparently normal (4)	14-42	Apparently normal tadpoles
Total (100)	(100)	(100)		

in vitro by Holtfreter,[4] by Hall,[5] and others.

The foregoing experiment was then twice repeated with the single difference that first distilled water, and then physiological salt solution, were substituted for tap water. There was no material difference in the results obtained.

The similarity of these effects to those observed by Keppel and Dawson suggested that abnormalities might result from direct diffusion of colchicine into the egg substance from the sperm water. To eliminate this possibility, portions of 2 testes were macerated in a very small amount (0.2 ml) of 0.04% colchicine solution. After 3 hours, enough tap water was added to reduce the concentration to $2.6 \times 10^{-4}\%$. The remaining portions of the same testes were macerated in the same amount of tap water. To this, after 3 hours, enough dilute colchicine solution was added to effect the same concentration, $2.6 \times 10^{-4}\%$. Eggs were then added at once to both suspensions. As a result of this procedure, only those eggs fertilized by sperm exposed to 0.04% colchicine developed abnormally. From this it is apparent that the effects were *not* produced by direct diffusion of colchicine into the egg substance from the sperm water.

Solution of the second problem, the nature of the role of colchicine in the production of these abnormalities, requires experimental investigation of a number of previous questions. Normal development is usually regarded as consisting of activities within, and interactions between, a self-perpetuating genetical enzyme system and the milieu in which it exists and acts. This indicates that in the present experiment colchicine probably chemically altered: either one or more reactants in the milieu; or one or more substances influencing these reactants; or both. As a first step in selecting among these possibilities, it seemed desirable to ascertain whether colchicine had acted by irreversibly altering something in the sperm prior to fertilization.

Accordingly the previous procedure was repeated. In this instance, however, after reduction of concentration from 0.04% to $2.6 \times 10^{-4}\%$, sperm were allowed to stand for 3 additional hours at room temperature before use. This was done in order to allow free colchicine to diffuse out of the sperm heads. Subsequent observation surprisingly revealed that eggs fertilized by such sperm developed as normally as eggs fertilized by untreated ones. Consequently, unless it be

[4] Holtfreter, J., *Arch. f. Entw-mech.*, 1931, **124**, 404.

[5] Hall, T. S., *J. Exp. Zool.*, 1942, **89**, 1.

supposed that extra standing rendered altered sperm completely incapable of initiating cleavage, which is for many reasons unlikely, it appears that in these experiments colchicine did *not* act by irreversible alteration of anything in the sperm prior to fertilization.

Summary. Eggs of the grass frog manifest various developmental abnormalities after fertilization with sperm previously exposed for 3 hours to 0.04% colchicine solutions. Experimental evidence is presented to show that this is not due to direct diffusion of colchicine into the egg from the sperm water nor to irreversible alteration of anything in the sperm prior to fertilization.

The author expresses thanks to J. Auer, M.D., Department of Pharmacology, St. Louis University School of Medicine, for his generous contribution of a considerable portion of his private supply of chemically pure colchicine alkaloid powder, a substance recently difficult to secure from the accustomed sources.

15418

The Citric Acid and Aconitase Content of the Prostate.

E. S. GUZMAN BARRON AND CHARLES HUGGINS.

From the Chemical Division, Department of Medicine, and the Department of Surgery, The University of Chicago.

The prostatic tissue has many properties of special interest. The human prostatic fluid is remarkable for its high content of citric acid and acid phosphatase, while the dog's prostatic fluid is poor in citric acid.[1-3] The growth of the tissue is greatly dependent on the sex hormones which also take part in the regulation of the oxidative phase of carbohydrate metabolism.[4] The human prostate is unable to utilize citric acid and α-ketoglutaric acid. The dog's prostate utilizes both acids, although not as much as the kidney or the heart. Synthesis of citric acid occurs in both tissues.[5]

The large amounts of citric acid found in human prostatic fluid seem to be produced by the prostate as indicated by the large citric acid content of the tissue. As can be seen in Table I, the citric acid content of human hypertrophic adenoma was about 645 mg per 100 g of fresh tissue, the values fluctuating between 1533 and 201 mg. The citric acid content in prostatic cancer was decreased by a factor of almost 10, to about 74.5 mg. The dog's prostate was found to be quite low in citric acid, about 8 mg per 100 g of tissue, *i.e.* 80 times less than the citric acid content of human prostate.

It seems that one of the causes for the large amount of citric acid in human prostate is the presence of powerful transaminations leading to the formation of oxaloacetic acid. The condensation of this dicarboxylic acid with pyruvic acid would give cisaconitic acid which in the presence of aconitase would be hydrated into citric acid. For the formation of citric acid by this process, the prostatic tissue would have to be rich in transaminase and in aconitase. Indeed there is vigorous transamination in the prostatic tissue.[5] Fig. 1 shows that the human prostate is also rich in aconitase, the dog's prostate containing only about half as much. Aconitase was extracted from the tissues by grinding with sand and 5 volumes of 0.1 *M* phosphate of pH 7.4. The suspension was centrifuged and the clear supernatant fluid was diluted so that it contained around 5 mg of tissue. To 4 cc of this solution was added 4 cc of 0.92 *M* cisaconitic

1 Schersten, B., *Skand. Arch. Physiol.*, 1936, **74**, Supp. 9.

2 Dickens, F., *Biochem. J.*, 1941, **35**, 1011.

3 Huggins, C., and Neal, W., *J. Exp. Med.*, 1942, **76**, 527.

4 Barron, E. S. G., and Huggins, C., *J. Urol.*, 1944, **51**, 630.

5 Barron, E. S. G., and Huggins, C., *J. Urol.*, 1946, **55**, 385.