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Resistance of Sperm of *Rana pipiens* to Hydrostatic Compression; Effect upon Embryonic Development.

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Various chemical and physical treatments have been applied to sperm, but only one of these, namely, radiation by X-ray or radium, has been very effective in inducing developmental changes after normal eggs have been fertilized with the treated sperm (Rugh[1]). Agents such as alcohol and other toxic substances, or such as abnormally low or high temperatures (Dunlay[2]) are difficult to use. Apparently the motility of the sperm is abolished by degrees of treatment which are insufficient to produce any marked changes in the nuclear complex of the gametes.

Three considerations suggested the use of hydrostatic pressure in an attempt to induce nuclear changes in the sperm: (1) No previous studies of the effects of pressure upon sperm have been reported; (2) It is known that high pressure can induce changes in the specificity of complex proteins such as viruses (Basset, Nicolan and Macheboeuf[3]) and antigens (Basset, Macheboeuf and Perez[4]) and therefore might conceivably be effective in inducing genic changes; and (3) In some previous work (Marsland and Brown,[5] and Pease and Kitching[6]) it has been observed that the motility of ciliated and flagellated forms is not abolished at pressures up to 600 atmospheres.

The sperm of *Rana pipiens* were obtained by excising the testes of recently hibernating frogs. Four to 6 of the intact organs, each from a different frog, were placed in a small (about 3 cc) glass test tube completely filled with 10% Holtfreter solution so as to exclude all undissolved air from the system. A rubber membrane served to seal the tube and to transmit the pressure from the surrounding Holtfreter solution which filled the bomb. To eliminate the possibility of toxic effects emanating from the rubber, the membrane was washed for a half hour in warm N/10 NaOH solution and rinsed

1 Rugh, Roberts, *Proc. Am. Phil. Soc.*, 1939, **81**, 447.

2 Dunlay, Neil S., *Biol. Bull.*, 1913, **25**, 213.

3 Basset, J., Nicolan, S., and Macheboeuf, M. A., *C. r. Acad. Sci.*, 1935, **200**, 1882.

4 Basset, J., Macheboeuf, M. A., and Perez, J. J., *C. r. Acad. Sci.*, 1935, **200**, 4960.

5 Marsland, D. A., and Brown, D. E. S., *J. Cell. and Comp. Physiol.*, 1936, **8**, 167.

6 Pease, D. C., and Kitching, J. A., *J. Cell. and Comp. Physiol.*, 1939, **14**, 135.

thoroughly in distilled water. Controls indicated that rubber so treated was non-toxic. In one experiment the sperm were liberated into the Holtfreter solution prior to the compression.

After compression, the sperm were used immediately to fertilize a minimum of 800 eggs which were obtained by the method of Rugh.[7] In most cases control fertilizations, utilizing sperm from the opposite testes of the same frogs, were carried out. The maximum available pressure, namely, 544 atmospheres (8000 lb/in.^{2}) was used in all of the experiments. The development of both the control and the experimental specimens was followed for 10 days, *i. e.*, until well after the hatching of the tadpoles.

The experimental results indicate that the sperm are quite immune to the pressures employed. In all cases they were normally motile after the compression period. Also the sperm retained their motility during the compression period, as could be seen when they were examined in the microscope-pressure chamber (Marsland and Brown[5]). There was no observable change in motility as pressure was being applied, and activity continued unabated during a compression period of 3 hours at 544 atmos. Apparently the motility of sperm is much less susceptible to the effects of pressure than is amoeboid movement (Marsland and Brown[5]), and protoplasmic streaming (Marsland[8]).

The compressed sperm gave the same fertilization results as did the controls (*i. e.*, practically 100%), and fertilization was followed by normal development well beyond the hatching stage. In the most prolonged experiment, the maximum pressure of 544 atms. was maintained continuously for a 3-hour period. Careful examination of the tail tips of tadpoles showed no appearance of haploid or other chromosome aberrations such as were obtained by X-ray treatment (Rugh[1]). A cytological study of the cells of some early embryos failed to reveal any abnormalities.

One experiment was done to determine if frequent rapid changes in the pressure level might have a greater effect than a continuously maintained high pressure. In this case during the last 2 hours of a 3-hour compression period, the pressure was dropped to 1 atms. at 5-minute intervals, and then immediately restored to 544 atms. The decompression occurred in less than 1 second and the subsequent build-up required about 5 seconds. Thus the total compression time was reduced by less than 3 minutes during the 24 successive decompressions. Nevertheless these sperm gave rise to a perfectly normal set of embryos and larvae.

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[7] Rugh, R., *Biol. Bull.*, 1935, **66**, 22.

[8] Marsland, D. A., *J. Cell. and Comp. Physiol.*, 1939, **13**, 23.

The suitability of pressure as a means of inducing genic or chromosomal changes in the sperm cannot be decided until experiments are done using higher pressures. However, pressure higher than 544 atms. is not available at present in our laboratory.

Possibly genic or chromosomal changes were not to be expected in the range of pressure which was used, despite the relatively long duration of the compression period. However, certain viruses do begin to undergo denaturation at a somewhat higher pressure, namely, 1000 atms., applied for 45 minutes (Basset, Wollman, Machelboeuf and Bardach[9]). On the other hand, a number of proteins retain their antigenic specificity at pressures up to 4000 atms. (Basset, Macheboeuf and Perez[4]) and a few do not appear to be denatured below 10,000 atms. (Basset, Lisbonne and Macheboeuf[10]).

Conclusion. Hydrostatic pressure of 544 atms. applied to frog sperm, either continuously, or in the alternating periods of compression and decompression, for a period of 3 hours, in no way alters the motility or the fertilizing power of the sperm. More than 5000 embryos resulting from such sperm and normal eggs, developed normally in all respects.

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Induction of Lymphomatosis in Mice Following Painting with 9:10 dimethyl-1:2 benzanthracene.

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There is sufficient evidence to indicate the existence of a direct relationship between carcinogenic agents and certain types of tumors in mice, *e. g.*, carcinoma of the lung, sarcoma, epithelioma. Given the hereditary predisposition to tumor formation, the application of certain carcinogens will hasten the appearance and in some instances

9 Basset, J., Wollman, E., Macheboeuf, M. A., and Bardach, M., *C. r. Acad. Sci.*, 1933, **196**, 1138.

10 Basset, J., Lisbonne, M., and Macheboeuf, M. A., *C. r. Acad. Sci.*, 1933, **196**, 1540.

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