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A Pressure Device for the Separation of Mammalian Spermatozoa from the Isolated Epididymis.

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During an investigation of the specificity of mammalian spermatozoa[1] it was desired to obtain a pure suspension of spermatozoa for purposes of chemical fractionation. As a very large quantity of material was needed, it was necessary to devise a means of separating them from the isolated epididymis with a minimum of labor. The following method, using a pressure device, has been found useful in rapidly separating the spermatozoa from a large number of the organs.

The pressure device consists of a foot-pedal which activates a pressure bulb fitted with 2 one-way valves in line, a water reservoir, and pressure tubing leading to a blunt No. 22 gauge hypodermic needle fitted with a shut-off valve.

The testes of bulls and rams were obtained from the abbatoir with all their coverings intact. After washing off all adherent blood clots, the cavity of the tunica vaginalis was opened and the cauda epididymis removed together with a long section of the vas deferens. These pieces of epididymis with the adherent vas deferens were placed in sterile saline until used.

The open end of the vas deferens was pulled over the blunt needle, and secured by means of a clamp, the jaws of which were covered with fine rubber tubing, to prevent cutting of the tissues and backflow. Pressure was then applied by means of the foot-pedal and water was injected into the epididymis. After injecting a small amount of water, the canal protruded in certain fields, one field appearing after the other over the shiny surface of the epididymis. When the distention became great enough to threaten rupture of the canal, an incision was made in the last field which filled up, more pressure was applied, and the spermatozoa were forced through the incision into a sterile vessel. The suspension of spermatozoa was then washed once with water. All of these manipulations were carried out with precautions to minimize contamination.*

[1] Henle, Werner, *J. Immunol.*, 1938.

* The technic used in obtaining spermatozoa from the epididymis of smaller animals such as guinea pigs, rats, and mice has been fully described elsewhere.[1]

This mechanical device might also be found useful in the exsanguination of organs.

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Crystalline Vitamin B-6.*

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Vitamin B-6 was designated by György[1, 2] as the "rat pellagra-preventing" principle present, as defined by his biological test, in such materials as yeast, liver, rice bran, etc. He indicated that this active substance had the properties of a nitrogen base.[3]

Starting with rice bran, we have been able to isolate the crystalline hydrochloride of a nitrogen base having the properties of Vitamin B-6 as defined by György. The hydrochloride is freely soluble in water and sparingly in alcohol and acetone. It is obtained as white platelets melting at 204-206°C. with decomposition. The chemical composition is being studied and will be reported at a later date.

The biological testing of our crystalline substance was carried out by Dr. W. L. Sampson of the Merck Institute of Therapeutic Research. The method used was the curative rat method as suggested by Moll.[4] In this procedure rats are maintained on a Vitamin B-6 deficient ration, similar to that used by György, except that a good grade of corn starch is substituted for rice starch. When the rats have developed severe symptoms of the deficiency, a single dose of the test material is administered by mouth. The effective dose is that amount which will produce a healing of the symptoms within a period of 14 days. Our crystalline preparation was tested by this method at dose levels of 0.100 mg., 0.050 mg., and 0.025 mg. The protocols are shown in Table I.

The degree of the symptoms is indicated by a scoring system similar to that given by Halliday and Evans.[5]

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1 György, P., *Nature*, 1934, **133**, 498.

2 Birch, T. W., György, P., Harris, L. J., *Biochem. J.*, 1935, **29**, 2830.

3 Birch, T. W., György, P., *Biochem. J.*, 1936, **30**, 304.

4 Private communication.

5 Halliday, N., and Evans, H. M., *J. Nutrition*, 1937, **13**, 657.