TECHNICAL PAPERS

Hypervolemia in Mice Bearing Transplantable Granulosa Cell Tumors[1]

JACOB FURTH and HARRY SOBEL

Cornell University Medical College and *New York Hospital, New York City*

It has recently been reported (*1*) that the livers of mice bearing transplantable granulosa cell tumors of the ovary are extremely congested and are greatly increased in weight. Microscopically, it is seen that the liver sinusoids are extremely distended with blood, so that their width is several times that of the cords of the liver cells. At the advanced stage of this condition the liver cells undergo atrophy; some vessels become thrombosed, and necrosis of liver tissue supervenes. The hyperemia is not restricted to the liver, for the adrenal, ovary, and spleen are sometimes also extremely congested; but no organ above the diaphragm has been noted to be involved.

The massive hyperemia suggested an increase in the blood volume. This was determined by adapting the exsanguination-perfusion technic and the Evans blue (T-1824) technic to the mouse.

The data, summarized in Table 1, indicate a huge increase in

TABLE 1

BLOOD VOLUME IN MICE

Mice	Exsanguination-perfusion technic			Dye technic		
	No. in group	Body weight %		No. in group	Body weight %	
		Extremes	Average		Extremes	Average
Normal	12	3.5– 6.6	5.2	9	9.0–12.7	10.9
With granulosa cell tumors	24	7.3–35.8	13.6	10	9.7–54.6	34.3
With other tumors	10	3.7–11.0	6.9	6	6.9–12.4	9.0

the blood volume of animals bearing granulosa cell tumors, heretofore unknown to occur in any condition. Mice bearing any of four different types of tumors of comparable mass and vascularization possess blood volumes that do not differ appreciably from those of normal mice. This huge increase in blood volume is apparent even after simple exsanguination,

The hematocrit values are normal, slightly increased, or decreased indicating that there is an enormous increase in both plasma and red cells.

There is a direct relationship between blood-volume values and congestive changes in liver as seen in sections. In mice with marked congestion, the blood-volume values, as determined by the exsanguination-perfusion technic, were 3.7–17.2 cc. (12.3–35.8 per cent of body weight); with moderate congestion, 2.0–9.4 cc. (7.3–21.3 per cent of body weight); and with slight congestion, 2.0–3.8 cc. (7.6–10.8 per cent of body weight), as compared with 1.3–2.1 cc. in normal mice and 1.2–3.8 cc. in mice bearing other tumors.

It is possible that these granulosa cell tumors secrete a substance, as yet unknown, or a substance already known, which, when produced in ever-increasing amounts as the tumor grows, causes the hypervolemia. Such a substance is being sought.

It is possible that the vasodilatation accompanying hypervolemia is caused by an excessive amount of the vasodepressor material described in *Science* by Shorr, Zweifach, and Furchgott (1945, **102,** 489) and that the phenomenon is a disturbance of the homeostatic vascular mechanism related to shock.

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Reference

1. FURTH, J., and BOON, M. C. *Proc. Soc. exp. Biol. Med.*, 1945, **58**, 112.

Streptomycin as an Aid in Isolating Influenza Virus[1]

ALBERT P. MCKEE and WM. M. HALE

College of Medicine, State University of Iowa

Hirst (*3*) used penicillin to thwart bacterial contamination in the isolation of influenza virus from unfiltered throat washings inoculated into the amniotic sac of the developing chick embryo. This same method was used by us to advantage in the type B influenza epidemic of November 1945.

Nose and throat washings obtained from three patients on November 20 were pooled, frozen on solid CO_2, thawed, ground in a mortar, and centrifuged. To each milliliter of unfiltered washings 500 units of penicillin were added, and 0.1 ml. of the mixture was inoculated into each of 24 twelve-day-old embryonated eggs. Of the 24 inoculated, 8 were alive after two days incubation. The live, embryonated eggs were tapped after two days incubation at 35° C. and again on the third day, and were found to contain influenza virus, type B, by Salk's (*4*) modification of the Hirst (*2*) hemagglutination and anti-hemagglutination test.

Because of the high death rate of the embryos experienced above, we added 1,000 units of streptomycin/ml. of washings in addition to the 500 units of penicillin. By use of both antibiotics we reduced our losses to less than 10 per cent of the inoculated embryos.

Subsequently both type A (isolated in Iowa City from 1943 epidemic) and type B (current strain) were tested in embryonated eggs to determine whether the streptomycin-penicillin combination interfered in any way with their propagation. We found as much virus produced in the presence of the antibiotics as with control eggs containing no streptomycin or penicillin.

Since that time we have used the streptomycin-penicillin

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