TECHNICAL PAPERS

Hypervolemia in Mice Bearing Transplantable Granulosa Cell Tumors[1]

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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

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Streptomycin as an Aid in Isolating Influenza Virus[1]

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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

[1] This investigation was aided in part by the Commission on Influenza Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army, Washington, D. C.

combination on a number of unfiltered specimens, including nose and throat washings, sputum specimens, and 10 per cent lung suspensions, when amniotic sac inoculations were desired. The death rate of the embryos and the incidence of positive cultures from embryonic fluids have been considerably lower when the two antibiotics were used than when penicillin alone was used. In fact, the occurrence of bacterial contamination when both antibiotics were used has been uncommon. One example has been encountered in which the combined antibiotics failed to protect the embryos in any degree. In this instance we inoculated embryonated eggs with a 10-per cent lung suspension from a human case of suspected influenzal pneumonia terminating fatally. All embryos died in 24 hours. The bacterial contaminant was a strain of *Pseudomonas pyocyanea*, which proved to be very resistant to streptomycin.

In some of our virus studies we have had occasion to pass mouse lungs at short intervals (24–48 hours) for a number of passages. Almost without exception we have encountered difficulty from bacterial pneumonias. However, when passages were made at 96-hour intervals, this difficulty was not encountered. We have assumed that these pneumonias arise as a result of washing bacteria from the upper respiratory tract of the mouse into his lungs as a result of the intranasal inoculation. The 24- and 48-hour intervals do not allow sufficient time for the mice to eradicate these organisms, and the rapid passages probably increase the virulence of these bacteria to the point at which they can kill the animal. Almost without exception the offending organism has been an alpha-hemolytic streptococcus or a small gram-negative rod (not *Salmonella*). Recently we have obviated the difficulty encountered in rapid lung passages by the addition of 500 units of penicillin and 1,000 units of streptomycin/ml. of 10 per cent mouse lung used for passage. The antibiotics and lung suspension are usually allowed to contact each other for about one hour before inoculations are made.

TABLE 1

CULTURES OF MOUSE LUNG

Time of sacrifice after intranasal inoculation	With antibiotics		Without antibiotics	
	Aerobic	Anaerobic	Aerobic	Anaerobic
24 hours	No growth	No growth	50 colonies*	32 colonies
48 hours	4 colonies	No growth	144 colonies	400 colonies

* The colonies consisted of nonhemolytic gram-negative rods, alpha-hemolytic streptococci, and anaerobic streptococci.

To check on the above technic we have inoculated two groups of mice (10 in a group) with 10 per cent normal mouse lung suspension intranasally. In one group the two antibiotics were added to the normal lung suspension before the inoculation. The other group served as a control. After 24 hours five mice from each group were sacrificed, their lungs pooled and ground to a 20-per cent suspension in saline, and one loopful of the suspension cultured aerobically and anaerobically. The remaining mice were tested in 48 hours. The results are shown in Table 1.

Addendum. Since submission of this article to the Surgeon General's Office for publication an article has appeared (*1*) in which the authors reported that they found streptomycin without toxicity for chick embryos and that there were no untoward effects on influenza virus, types A and B.

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Meteorite Impact Suggested by the Orientation of Shatter-Cones at the Kentland, Indiana, Disturbance

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A large quarry about two miles east of Kentland, Newton County, Indiana, exposes the center of a well-known local area of intensely deranged Paleozoic beds in a region of essentially flat-lying strata. It is generally accepted that a violent natural explosion in the geological past formed this (*3*, *4*) and similar disturbances (*2*, *5*). The application of violent shock in the formation of the Kentland disturbance is indicated in part by the jumbling of the strata, the shattering of the limestones, and the pulverization of the sand grains of the St. Peter sandstone. Although there is no indication of the presence of igneous material or of hydrothermal alteration, Bucher (*3*) and Shrock (*4*) have ascribed the origin of this disturbance to a deep-seated explosion of gases derived from an igneous intrusion. The observation made recently by the present writer may invalidate such a cryptovolcanic explosion hypothesis or any other suggested mode of origin that involves a deep-seated force acting from below the strata.

Prominently developed in the limestones at the Kentland disturbance are cup-and-cone structures called "shatter-cones," "pressure-cones," or "shear-cones," which were apparently produced by the explosive shock which formed the disturbance. These curious shatter-cones are present in two other explosion disturbances of the Kentland type but are not reported from other types of geological disturbances. If well developed, these structures consist of a large primary cup-and-cone which has a surface grooved in such a manner as to appear as a series of smaller secondary, overlapped and imbricated half-cones, with the apexes of these secondary features pointing toward the apex of the primary cup-and-cone. This convergingly-grooved surface is a fault surface similar to the parallel-grooved slickensided surfaces common in fault zones. An examination of the cup-and-cone surface and of the base of some of these features reveals that the cup is displaced relatively downward, *i.e.* away from the apex, with respect to the cone, so that these are normal faultlets rather than thrust faultlets. By applying Hartman's law, which states in part that under nonrotational forces the acute angle formed by shear planes in brittle material is bisected by the axis of maximum stress, it is apparent that the axis of such a primary cup-and-cone is also the axis of maximum stress. An examination of many of these structures at Kentland revealed that the axes of the primary cup-and-cones are invariably oriented *normal* to the bedding and the apexes point toward the *top* of the bed. The common orientation of the apexes indicates