Effect of Homologous and Heterologous Tissue on Mitosis in Heterozygous and Inbred Mice. (17501)

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(Introduced by Elton S. Cook)

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A significant inhibition of mitotic activity in the epithelial cells in the crypts of Lieberkühn in *dba* mice was produced by repeated subcutaneous injections of an aqueous alcohol-treated extract of beef spleen.(1) In the present experiments the effect of a single subcutaneous injection of splenic pulp of homologous and heterologous origin on mitotic activity of the cells in the crypts of Lieberkühn has been investigated in a highly inbred and a heterozygous strain of mice.

Experimental. Twelve *dba* line 1 mice from the Jackson Memorial Laboratories and 9 of the Rockland Farm all-purpose strain were employed in this study. The mice were approximately 6 to 8 weeks old and were allowed food and water *ad libitum*. Spleens were removed aseptically from donor mice of the same strains, pulped by passage through a fine meshed wire screen, and diluted 1:1 with Ringer's solution. A single subcutaneous injection of 0.5 cc was made into the groin of each experimental animal, and 7 days later the animals were killed, always at the same hour of the day in order to avoid introducing the diurnal variable in the mitotic cycle.(2) At autopsy a short segment of the small intestine located 20 mm distal to the pylorus was removed, fixed in Bouin's solution, dehydrated and embedded in paraffin. Histologic sections 8 mu in thickness were stained with hematoxylin.

Mitotic figures including those from late prophase to late anaphase were counted in the epithelium of the crypts of Lieberkühn in cross sections of the gut. Ten sections from progressive levels of the segment sufficiently separated to rule out counting the same figure more than once were used from each animal, and 50 fields in each of these sections were studied at a magnification of 970, a total of 10,500 fields being explored.

Two untreated groups of animals consisting of 5 Rockland Farm (Group I) and 5 *dba* line 1 (Group II) mice served as controls. The effect of spleen pulp from the Rockland Farm mice on mitosis in 4 Rockland Farm mice (Group III) and 5 *dba* line 1 mice (Group IV) was studied, and also the effect of *dba* spleen pulp on mitosis in *dba* gut epithelium (Group V). There were not sufficient *dba* animals to study the effect of *dba* spleen pulp on mitosis in Rockland Farm mice.

The significance of the data obtained from the different groups was determined by the formula(3) designed for small samples:

$$t = \frac{M_1 - M_2}{\sqrt{\left(\frac{N_1\sigma_1^2 + N_2\sigma_2^2}{N_1 + N_2 - 2}\right)\left(\frac{N_1 + N_2}{N_1 N_2}\right)}}$$

Levels of significance are expressed in percentage.

Results. No significant difference in mitotic activity in normal control mice of the highly inbred *dba* strain and the heterozygous Rockland Farm mice was found (Groups I and II, $t = 0.216$; significance level 20%). Homologous spleen in *dba* mice had no effect on the number of mitotic figures (Groups I and V, $t = 1.97$; significance level $> 20\%$) while homologous spleen in Rockland mice appeared to reduce the mitotic count slightly (Groups II and III, $t = 1.67$; significance level $< 5\%$). When splenic pulp from Rockland Farm mice was implanted in the *dba* mice, however, there was a marked increase in the number of mitotic figures observed (Groups I and IV, $t = 3.42$;

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3. Lindquist, E. F., *A. First Course in Statistics*, Houghton Mifflin Co., Boston, 1942, p. 138, p. 240.

TABLE I.
Effect of Homologous and Heterologous Spleen Pulp on Mitosis in Intestinal Epithelium.

Group	No. mice	Mitotic counts: Group avg ± S.D.	Mitotic counts: Range
I. Control *dba*	5	1.34 ± 0.250	(0.86–2.10)
II. Control Rockland	5	1.33 ± 0.267	(0.68–1.80)
III. Rockland spleen in Rockland	4	1.23 ± 0.295	(0.90–2.04)
IV. Rockland spleen in *dba*	5	1.55 ± 0.350	(0.82–2.46)
V. *dba* spleen in *dba*	2	1.21 ± 0.236	(0.80–1.74)

significance level 0.1%).

Discussion. The stimulation of mitotic activity obtained with heterologous tissue in these experiments tends to confirm the results of Breuhaus and McJunkin(4) using intraperitoneal injections of macerated rat kidney in normal and unilaterally nephrectomized rats.

Our results would indicate that a foreign tissue can influence mitotic activity as judged by the reaction of the epithelium in the crypts of Lieberkühn in the small intestine of the mouse, whereas a homologous tissue may not do so, as illustrated by the lack of significance of the data from *dba* mice receiving *dba* tissue. The borderline significance level for homologous tissue in Rockland mice, however, may possibly be attributed to the fact that this is a heterozygous strain and there would be a greater degree of difference between tissues in animals of this stock than between those in animals of the highly inbred *dba* line 1 strain.

Summary. The subcutaneous injection of spleen pulp from heterozygous Rockland mice into homozygous *dba* line 1 mice caused a significant increase in the mitotic activity in the epithelium of the crypts of Lieberkühn, whereas the injection of the spleen pulp from *dba* mice into mice of the same strain was without significant effect, and the injection of Rockland spleen into Rockland mice caused a mitotic decrease of borderline significance.

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Choline Deficiency in the Hamster. (17502)

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In a previous communication(1) it was noted that there is a correlation between the occurrence of fatty livers in choline deficient animals and the ability of the livers of normal animals of a given species to oxidize choline. Thus, rats,(2), mice,(3) and dogs(4) develop fatty livers on choline deficient diets and their livers possess an active choline oxidase.(5) In contrast, the guinea pig does not develop a fatty liver while living on a choline deficient diet(1) and the livers of this species are devoid of choline oxidase activity.(5) In the present work it has been found that the liver of the normal hamster shows only moderate choline oxidase activity and that liver fat accumulation in this species is less than that

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4. Best, C. H., Ferguson, G. C., and Hershey, J. M., *J. Physiol.*, 1943, v79, 94.

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