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Storage of Carmine in Mice of Inbred Strains.

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Numerous observations have been reported which suggest a relationship between reticulo-endothelial tissues and the development and growth of malignant tumors (lit. cf.).[1] However, discrepant findings and controversial interpretations have as yet not permitted the formulation of a definite concept.

In the present study the attempt was made to approach this problem by making use of the great variation in spontaneous tumor development exhibited by certain inbred mouse strains. For this purpose, animals of the C3H strain with high mammary cancer incidence and animals of the low-cancer strain C57B were employed. The reticulo-endothelial activity of these animals was assayed on the basis of their ability to store lithium carmine.

Carmine (Merck & Co.) was dissolved in a saturated aqueous solution of lithium carbonate to the desired concentration by boiling gently for 3 minutes, followed by filtration through glass wool. Two, 3 and 4% solutions were employed. They were prepared within the 24 hours prior to use and sterilized in the autoclave for 20 minutes (pressure 10-15 lb). The carmine solutions were injected intraperitoneally with concentration and dosage varying in the 5 experimental groups, as shown in Table I. In the first 3 experiments the animals were sacrificed 48 hours after injection, in the last 2 after 24 hours. Sections of liver and spleen were fixed in 10% formalin; in some groups also skin, kidneys and lungs were preserved. Sections of 6 to 8 micron thickness were cut from the formalin-fixed and paraffin-blocked organs. Of each block, a hematoxylin-eosin stained section and a cleared unstained section were prepared. For general observations, the stained sections were studied. For detailed study of the carmine storage the unstained sections were found best suited.

Particular attention was given to the dye granules taken up by the Kupffer cells in the liver. The over-all impression was that of a more marked storage in Kupffer cells of C57B animals as compared with the findings in C3H mice. In order to express these relations in a roughly quantitative manner, an estimation of the amount of storage was carried out by counting the storing Kupffer cells (SKC) in at least 10 high-power fields and by determining the average of dye-laden cells in each animal. As seen in Table I, this crude quantitative estimation confirmed the impression obtained from general study of the slides.

In all of the 5 experiments, the average numbers of SKC observed in C57B mice exceeded those found in C3H mice. In view of the rather small number of animals making up the single experiments, it is, however, also necessary to analyze the values of SKC in the individual animals. Such an analysis shows that in Experiment I 4 out of 5 C57B mice presented markedly higher SKC values than the 4 C3H mice examined under the same conditions. In Experiment II, 4 out of 6 C57B animals surpassed 6 C3H mice in storing ability. In Experiment III, 1 of the 2 C57B mice showed considerable increase, the other slight increase of SKC as compared with 6 C3H mice. In Experiment IV, the SKC values of 5 out of 6 C57B animals were greater than those obtained in 6 simultaneously tested C3H animals (2 of the latter died 5 hours after the injection of the dye, and hence the small extent of storage probably was due to insufficient time elapsed). In Experiment V, 6 of 7 C57B mice presented higher SKC values than were found in 11 C3H animals under the same conditions. In

[1] Stern, K., and Willheim, R., *The Biochemistry of Malignant Tumors*, Brooklyn, Reference Press, 1943, pp. 696-745.

TABLE I.

			C3H			C57B		
				SKC*			SKC*	
Exp. No.	Carmine conc. and dosage	Time interval in hrs	No. of animals	Values	Mean	No. of animals	Values	Mean
I	3% 0.5 cc (15 mg)	48	4	31		5	54	
				38			67	
				41	38		67	69
				43			76	
							82	
II	2% 0.25 cc (5 mg)	48	6	16		6	22	
				22			35	
				22	25		50	50
				23			60	
				32			62	
				32			71	
III	2% 0.5 cc (10 mg)	48	6	13		2	27†	
				15			41	
				20	19			34
				20				
				21				
				22				
IV	2% 0.5 cc (10 mg)	24	6	5‡		6	24	
				5‡			53	
				10	13		60	69
				10			73	
				19			92	
				26			109	
V	4% 0.5 cc (20 mg)	24	11	10		7	31	
				15			51	
				17			57	
				22			62	
				28			63	
				33	29		64	56
				37			67	
				37				
				38				
				39				
				39				

* Storing Kupffer cells.
† Animal died after 30 hr.
‡ Animal died after 5 hr.

all, 20 out of the 26 examined C57B mice surpassed in carmine storing activity the 33 simultaneously tested C3H mice.

In addition to these quantitative findings, a qualitative difference was frequently noted: finely granular and pale red carmine deposits prevailed in 28 of the 33 C3H mice, with only 5 of them showing dark red, coarsely granular storage, while among the 26 C57B mice 14 presented dark red, coarse granules and 12 exhibited pale red, fine granules. In general, particle size and color intensity tended to increase with the concentration of dye used, though Table I shows that no exact parallelism existed between dye dosage and SKC values. In livers of C3H mice frequently diffuse distribution of dye in sinusoids was noted. Staining of parenchymatous liver cells (diffuse cytoplasmic stain and darker nuclear stain) occurred in both strains only in isolated areas of necrosis encountered infrequently. The intravital carmine staining of liver cells reported by Kiyono[2] was accomplished only by repeated dye injections.

No influence of age or sex was apparent in

[2] Kiyono, K., *Die vitale Karminspeicherung*, Jena, Gustav Fischer, 1914.

relation to the findings just described. The age of the animals ranged from 7 weeks to 10 months; for each experimental group animals of similar age were selected from both strains. The sex distribution was as follows: 17 males and 9 females among the C57B animals; 14 males and 19 females among the C3H animals.

In addition to the liver, the carmine storage was studied in the spleens of all animals. Here, too, a different behavior of the two strains, though less constant and striking, was noted: among C57B animals storage was absent in 2, slight to moderate in 17, and marked in 7; among C3H animals storage was absent in 12, slight to moderate in 21, and marked in none. The storage in the spleen was found mainly in the perifollicular reticulum cells and, to a lesser extent, in littoral cells of the sinuses. Thus halo-like formations around the follicles, consisting of dye-laden cells, resulted from the storage, a distribution which did not lend itself readily to quantitative evaluation. The dye storage in the renal epithelium and the dye excretion within the tubules conformed to the classic descriptions[2] and no difference between the two strains was detected. Likewise no appreciable difference was observed regarding dye deposits in skin and lungs, which were examined in some groups of animals.

The fact that in these experiments the liver, *i.e.*, the storage in Kupffer cells, presented the most marked difference between the two strains may be due to the route of administration employed, namely, the intraperitoneal injection of carmine. In this procedure, most of the dye is probably absorbed by serosal lymphatics and thus, by way of the portal vein, reaches the liver first. Intravenous injection, which was not used in this study, may possibly lead to different findings.

In previous work at the State Institute for the Study of Malignant Diseases, Buffalo, N.Y.,* similar experiments were carried out by comparing the carmine storage in C57B mice and in animals of the Marsh-Albino strain. Six animals of each strain were examined. They presented a marked difference in their dye storage in Kupffer cells and in the spleen: heavy, coarsely granular storage was obtained in 5 of the 6 C57B mice and only in 1 of the 6 MA mice.

The C3H and the MA strains are both known for the high incidence of spontaneous mammary carcinoma in the females. On the other hand, this type of tumor is exceedingly rare in the C57B strain. In the latter, the total incidence of all epithelial and non-epithelial spontaneous tumors is around 20% according to studies and records of the Jackson Memorial Laboratory.[3] At this time it would be premature to speculate on a possible relationship between susceptibility or resistance to spontaneous cancer development, on the one hand, and the reticulo-endothelial activity of the strain, as expressed in dye storage, on the other. In order to establish such a relationship it will be necessary to extend this work to a number of additional inbred strains with varying rates of spontaneous tumor development.

Summary. The storage of intraperitoneally injected carmine in Kupffer cells of the liver and in the spleens was examined in mice belonging to two inbred strains. Quantitative and qualitative differences were observed which indicated an inferior dye-storing ability of C3H animals as compared with C57B mice.

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3 Staff of the Jackson Memorial Laboratory, *Biology of the Laboratory Mouse,* The Blakiston Company, Philadelphia, 1941, p. 266.