Effect of Jensen Sarcoma upon the Reticuloendothelial System of Rats of Different Ages. (29005)

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When the phagocytic index of the reticuloendothelial system was measured by the rate of carbon clearance from the blood, the clearance rate decreased in older rats(1) and mice (2). It has also been observed that the Calmette-Guérin bacillus produced a smaller increase in the phagocytic capacity of older mice as compared to younger mice(2). Since spontaneous tumors usually appeared in older animals and only a minimal elevation of clearance rates was observed, it seemed possible that this might be partially explained by the inability of the older animal to respond(2). Following treatment with *Mycobacterium phlei* the phagocytic index was found to be greater in mice 6 months of age than those 1, 3, 9 or 12 months(3).

In the experiments with the Jensen tumor the clearance rate of carbon was elevated at an earlier period of tumor growth in the older animals than in the younger tumor-bearing rat. This would suggest that the type of stimulus applied may be important in determining the response of the reticuloendothelial system in rats of various ages. The older animals will give a faster rate of clearance than younger animals with some types of stimulation.

Materials and methods. Six groups of female Holtzman rats were used in these experiments. The first 5 groups were 2-, 3-, 4-, 5- and 6-month-old virgin females at the time of tumor inoculation. The last group of animals were discarded breeders 12 months of age or older. The average initial body weight in the various groups at time of tumor inoculation was 181, 201, 239, 258, 276, and 294 grams, respectively. The tumors were transplanted by an intramuscular injection of the Jensen sarcoma into both *rectus femoris*.

The activity of the reticuloendothelial system was determined by slight modifications of the method of Biozzi *et al*(4). A suspension of carbon (C 11/143a)† was injected into the femoral vein at a dose of 16 mg/100 g body weight. The rats were kept under light anesthesia during administration of carbon and sampling of the blood. At appropriate intervals blood was collected from the tail vein, and a 0.02 ml sample was pipetted into 5 ml of hemoglobin diluent—1 g $NaHCO_3$, 50 mg KCN, and 200 mg $K_3Fe(CN)_6$ diluted to 1 liter. The concentration of carbon was determined by its absorption at 540 mμ in a Beckman DU spectrophotometer. The blanks were samples of blood obtained prior to the injection of carbon. Blood carbon concentration decreased according to an exponential function of time expressed as follows:

$$\log C_1 - \log C_2/T_2 - T_1 = K$$

C_1 and C_2 were the concentrations of carbon in the blood at times T_1 and T_2, respectively. K, which expressed the phagocytic activity of the reticuloendothelial system for the injected dose, was called the phagocytic index.

It has been shown that the phagocytic index depends on the weights of liver and spleen(4). This allows the use of a corrected phagocytic index (α) expressed as:

$$\alpha = \frac{W}{WLS}\sqrt[3]{K}$$

W is body weight and WLS the sum of weights of liver and spleen.

Another method of correcting the phagocytic index has been proposed by DiCarlo *et al*(3) and is expressed K_{LS}. The formula is $K_{LS} = 10^3 K \frac{LS_c}{LS_E}$ where K_{LS} represents the relative phagocytic activity of liver and

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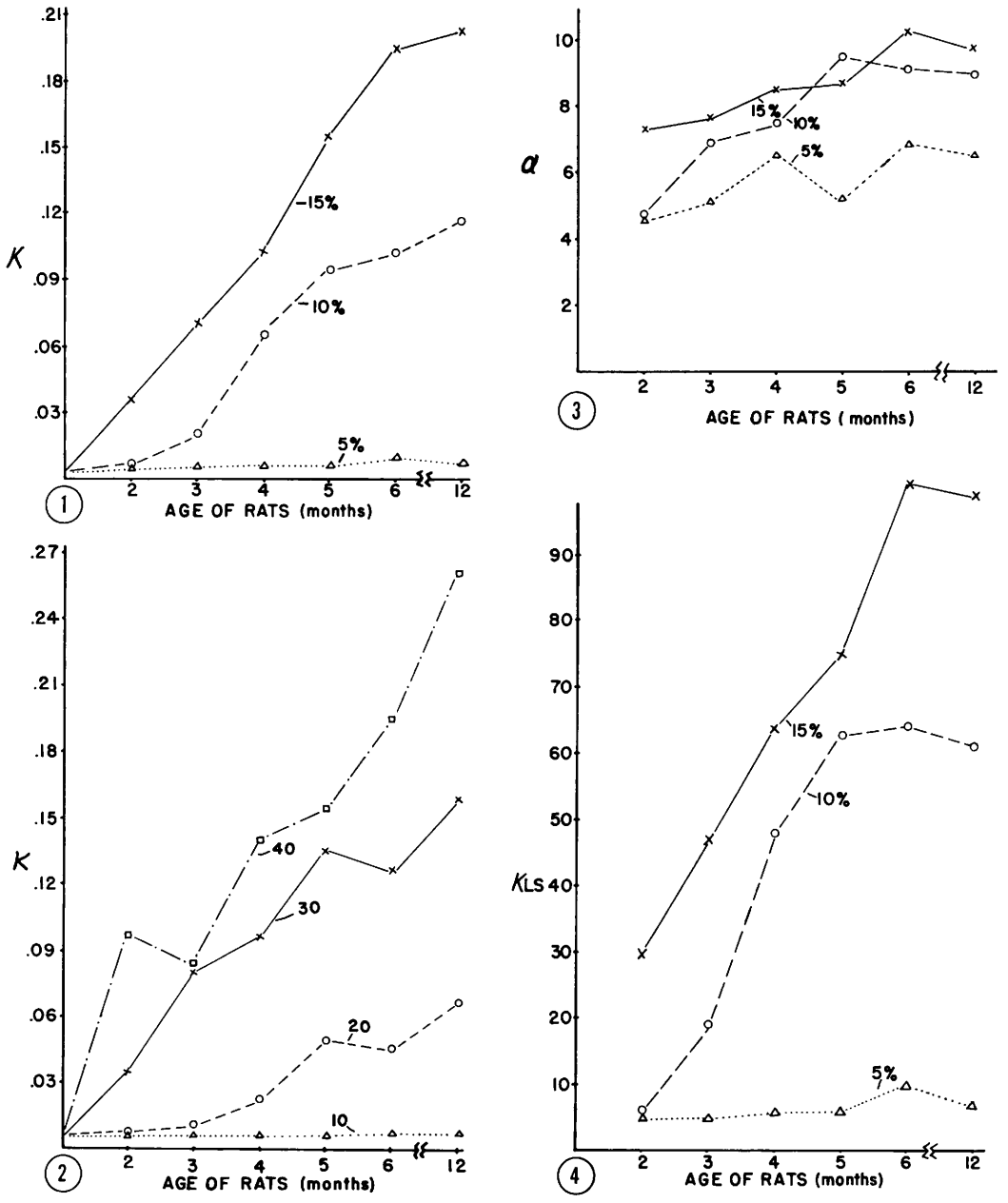

FIG. 1. Phagocytic index of rats of various ages during growth of the Jensen sarcoma. Dotted line represents animals which have tumors that average 5% of total weight (range 3-7%). Dashed lines represent tumors that average 10% of total body weight (range 8-12%) and solid line 15% (range 13-17%). Each point is average phagocytic index of 3-10 animals.

FIG. 2. Phagocytic index of rats of various ages during growth of Jensen sarcoma. Grouped by actual tumor weight: 10 (tumors weighing 6-15 g), 20 (tumors weighing 16-25 g), 30 (tumors weighing 26-35 g) and 40 (tumors weighing 36-45 g). Each point is average phagocytic index of 2-8 rats. Total number 88.

FIG. 3. Corrected phagocytic index of rats of various ages bearing Jensen sarcoma. Compared at various tumor weights which are expressed as percentage of total body weight of animal.

FIG. 4. Relative phagocytic activity of rats of various ages bearing Jensen sarcoma compared at various tumor weights which are expressed as percentage of total body weight of animal. See *Methods* section for meaning and method of calculating K_{LS}.

spleen, LS_c is the average per cent body weight of liver and spleen of a control group at a particular age, and LS_E represents the average per cent body weight of liver and spleen of tumor-bearing animals at a particular age.

Results and discussion. The effect of the Jensen sarcoma upon the phagocytic index of rats of different ages is shown in Fig. 1. The range of K values for normal rats was .004 to .011 with a mean of .006. There was a slight but not significant tendency for the younger normal animals to have a faster rate of clearance. When implanted with the Jensen tumor the phagocytic index did not increase in any of the age groups until the tumor was 7-8% of total body weight. With a slightly larger tumor (8-12% of body weight), the phagocytic index showed a marked increase in the older animals but little or no change in the younger animals. All of the tumors in the various groups grew to 15% of the total body weight in about 2 weeks with no significant difference in the rate of growth between age groups. The 2-month-old rats failed to show an increase in rate of carbon clearance until the tumor was almost 15% of total body weight. As the animal aged the rate of carbon clearance was faster at a smaller-tumor to body-weight ratio.

The older animals were heavier and had larger tumors for a given-tumor to body-weight ratio. A comparison was, therefore, made among the different age groups using actual tumor weight (Fig. 2). Even when plotted in this way, the older rats with 10 to 40 g tumors showed a faster rate of clearance than the younger animals.

Most of the carbon removed from the blood was found in the liver or the spleen, and these organs were heavier in the older tumor-bearing rats. The phagocytic index was corrected for the weight of the liver and spleen as indicated in Fig. 3. The corrected phagocytic index also demonstrated that when the tumors were small there was a progressive increase in carbon clearance rate with the age of the tumor-bearing host. Correcting for liver and spleen weights by the method of DiCarlo *et al*(3) gave a marked increase in the older rats bearing tumors which were 10 to 15% of total body weight (Fig. 4). The groups with tumors which average 5% of the body weight showed very little effect produced by the age of the animal when calculated as K_{LS}, but there was an increase with these small tumors when α values were used (Fig. 3).

Tumors developed in all 74 rats inoculated at 2 or 3 months of age with the Jensen sarcoma. No regressions were observed among the 34 animals which were followed for survival. One hundred and twenty-seven animals 4 months or older were inoculated. Of this number 97 developed palpable tumors. Forty-one of these rats were followed for survival and 14 regressions were observed. The significance of this apparent correlation between early reticuloendothelial stimulation during tumor growth and survival is still not clear. Perhaps both groups of data indicate that the Jensen sarcoma was not truly compatible in the older Holtzman rat. The rats in which tumors regressed, and also those which failed to produce tumors after inoculation, did not develop tumors after repeated implantation with the Jensen tumor. Old, Clarke and Goldsmith(5) reported no apparent correlation between degree of reticuloendothelial stimulation and ultimate survival of tumor-bearing animals. Many tumors have been reported which show maximal elevation of clearance rates during the early phase of tumor growth without resulting in appreciable tumor regression(2,5).

Therefore, it was not intended that these results should suggest a correlation between reticuloendothelial stimulation and survival during tumor growth, or that all tumors will produce greater reticuloendothelial stimulation in older animals. What was suggested was that the reticuloendothelial system in the older animal was capable of a greater response than that of the younger animal to this stimulation. It may be necessary, therefore, with each type of stimulus applied to the reticuloendothelial system, to evaluate the effect of the age of the animal.

Summary. Rats 2, 3, 4, 5, 6 and 12 months

old were inoculated with the Jensen sarcoma and the phagocytic index of the reticuloendothelial system was measured at various stages of tumor growth. The older animals bearing the Jensen tumor produced more and an earlier stimulation in the rate of carbon clearance than the younger tumor-bearing animals.

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Platelet Size in Relation to Platelet Age. (29006)

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In a study of the *in vivo* aging of rat blood platelets, the relative sizes of platelets of young, old, and normal populations were compared by measuring with a cathetometer the volumes of packed platelets in microhematocrit tubes; it was found that platelet size decreases with age(1). The purpose of this study was to investigate the frequency distributions of platelet sizes in populations of varying age using a Coulter Electronic Particle Counter with a particle size distribution plotter attached(2,3).

Methods. Blood was drawn from the abdominal aorta of anesthetized male Sprague-Dawley rats into cold, siliconed 20-ml syringes containing 1 ml of a 1% solution of filtered ethylenediaminetetraäcetic acid (EDTA) in 0.7% saline. All diluents used were filtered through a filter having 0.45 μ pore size. A standard volume of 10 ml of blood was collected from each rat and was diluted with 6.25 ml of filtered saline to obtain a better yield of platelets. The blood-saline mixture of each rat was equally divided into two 15-ml conical centrifuge tubes, and the platelets were separated by differential centrifugation in a refrigerated centrifuge (5°C). After a slow centrifugation of the blood at 107 $\times$ *g* (750 rpm in an International Refrigerated Centrifuge, model PR-2) for 40 minutes, the platelet-rich plasma-saline was removed and spun more rapidly at 760 $\times$ *g* (2,000 rpm) for 30 minutes to obtain a platelet button. The platelets were then resuspended in 0.9% saline to give concentrations of 9,747 to 37,030 platelets/50 μl. (The standard volume of suspension pulled through the counting aperture was 50 μl when making numerical counts.) Platelets that had been separated from blood and resuspended in saline were used in these experiments rather than diluted platelet-rich plasma because our own work and that of others(4) has indicated that, in addition to platelets, the latter may contain unidentified small particles that affect platelet counts and possibly size distribution curves.

Populations of young platelets were obtained from rats that were recovering from thrombocytopenia induced with antiplatelet serum(1). Four hours after injection of the antiplatelet serum there were no circulating platelets. At 24 hours the platelet count was approximately 4,000/mm^3, which was too few for satisfactory collection. Platelets were collected and sized 2, 3, 4, 5, 6, and 7 days after antiserum injections. Platelets collected 2 days after antiserum injection, therefore, had an age range of 0-2 days.

Populations of old platelets were prepared

* Operated by Union Carbide Corp. for U. S. Atomic Energy Commission.