Effect of *Mycobacterium phlei* upon Reticuloendothelial System of Mice of Different Ages. (28129)

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A previous report(1) described the bimodal stimulation of phagocytosis elicited in the mouse by a single injection of live *Mycobacterium phlei* Halpern. Earlier, Boehme and Dubos(2) and Boehme(3) showed that administration of *Salmonella abortus equi* endotoxin and *Mycobacterium fortuitum* Penso to mice also produced bimodal responses in the rate of clearing carbon from circulation. Almost identical data were obtained from *Mycobacterium phlei*(1) and the endotoxin(3) under the experimental conditions employed. Earlier limited studies indicated that older mice executed phagocytosis slower(4) and were less responsive to RES stimulants(2,3,4) than younger animals.

The present report describes a study of the normal rate of phagocytosis of mice of various ages (1 to 12 months) and the response of such animals to intravenous administration of *Mycobacterium phlei* Halpern. This investigation provided an opportunity to evaluate the "corrected phagocytic index" α as a general manner of expressing phagocytic efficiency by taking hepatosplenomegaly into account(5).

Materials and methods. The global phagocytic index K and the "corrected" phagocytic index α were determined by the carbon clearance procedure of Biozzi *et al.*(5). "Pelikan" India Ink C11/1431a (Günther Wagner, Hannover, Germany) was employed at a level of 160 mg of carbon per kg body weight. White female ICR strain mice (Millerton Research Farm, Millerton, N.Y.) were used throughout the investigation. The groups of mice were 1, 3, 6, 9 and 12 months old at time of treatment.

Thirty mice of a given age were handled at one time. Phagocytic indices (K and α) were determined on 5 untreated mice and *Mycobacterium phlei* Halpern (6 mg dry weight/kg body weight) was administered to the remainder. Phagocytic indices were determined on groups of 3-5 mice after 6-8 time intervals post administration. A control group consisting of 30 untreated 1 month old mice was tested by the carbon clearance method at the same time intervals. Three to 5 months later, the entire experiment was repeated. Fig. 1 and 2 were constructed from average values.

Results and discussion. With untreated control mice, no significant changes in global phagocytic index (K) were obtained between the ages of 1 to 12 months (Fig. 1). During this period the grand-mean K was 0.011 with a standard error of $\pm$ 0.0003. The calculated confidence limits (P = 0.99) ranged from 0.0093 to 0.0129 when number of mice per mean K at each age was 9. A mean K of 0.013 or greater indicated significant RES stimulation. Following treatment with *Mycobacterium phlei,* the K values for both peaks were significantly increased ($p<0.01$). Comparison of the 2 peak K values at each age indicated no significant difference (at $p>0.01$) between the first and second peaks at any given age. The over-all mean K elicited by administration of *Mycobacterium phlei* was 0.065. The calculated confidence limits (P = 0.99) ranged from 0.042 to 0.088. Comparison of the composite peak K value for each age (the mean of peaks 1 and 2) indicated that there was significantly greater than average RES stimulation at 6 months of age (K = 0.089). Each other composite peak K value was well within the range of 0.042 to 0.088.

Another noteworthy feature of the results depicted in Fig. 1 is that the 6 and 9 month old mice showed the maximum response 2 days after receiving the stimulant whereas the groups of 1, 3 and 12 month old mice reached peaks after only 1 day. In all 5 groups of mice, the second peak occurred at 17 to 22 days post administration. Between the 2 peaks, the treated 1 and 3 month old mice displayed phagocytic indices only a lit-

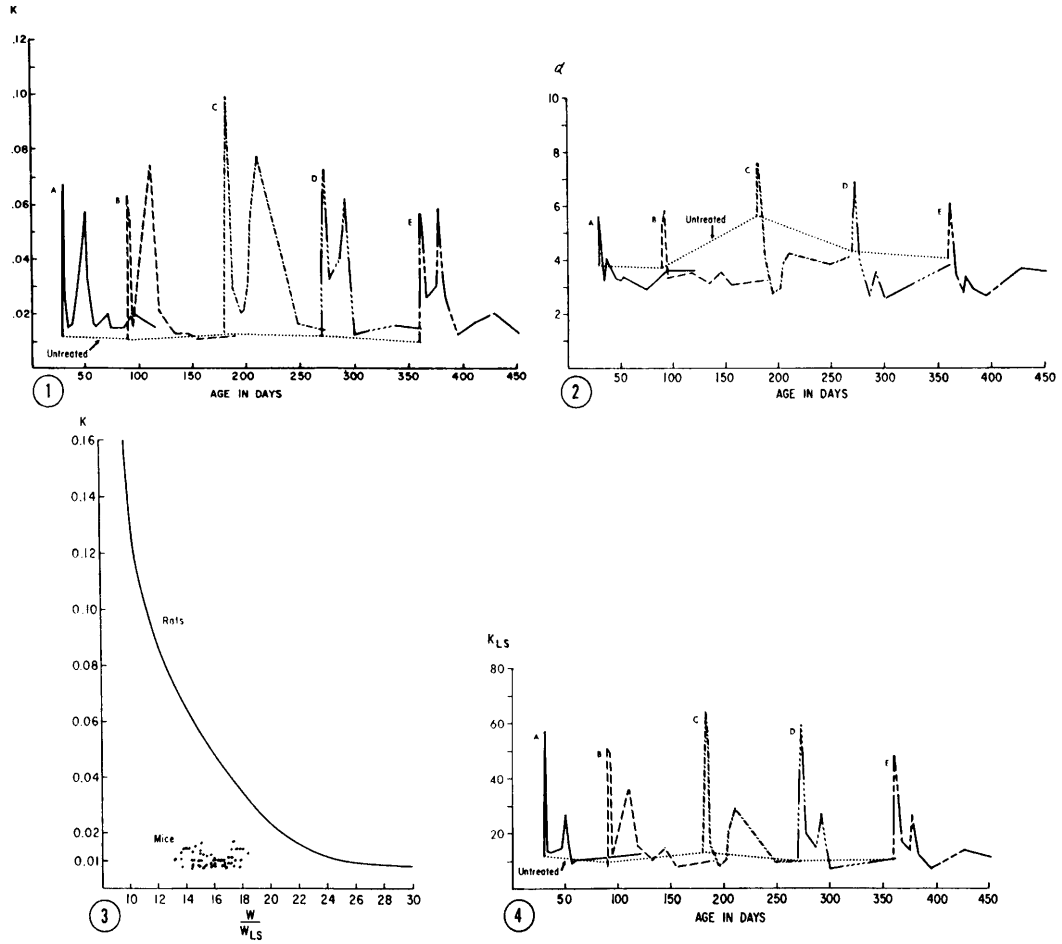

FIG. 1. Global phagocytic index K of untreated 1-, 3-, 6-, 9- and 12-mo-old mice and of groups of mice given single intravenous injections of *Myco. phlei* Halpern at A—1 mo of age, B—3 mo of age, C—6 mo of age, D—9 mo of age, and E—12 mo of age.

FIG. 2. "Corrected" phagocytic index α of untreated 1-, 3-, 6-, 9- and 12-mo-old mice and of groups of mice administered single intravenous injections of *Myco. phlei* Halpern at A—1 mo of age, B—3 mo of age, C—6 mo of age, D—9 mo of age, and E—12 mo of age.

FIG. 3. Comparison of K and W/W_{LS} values found for untreated rats by Biozzi *et al.*(5) with the points obtained from control mice during the present study.

FIG. 4. Phagocytic index of the liver and spleen K_{LS} of untreated 1-, 3-, 6-, 9- and 12-mo-old mice and of groups of mice administered single intravenous injections of *Myco. phlei* Halpern at A—1 mo of age, B—3 mo of age, C—6 mo of age, D—9 mo of age, and E—12 mo of age.

tle greater than the untreated control animals. With advancing age, the return to normal became less complete; the indices of 9 and 12 month old mice which received *Mycobacterium phlei* returned only about halfway to normal low level between peaks.

Fig. 2 summarizes values calculated for the "corrected" phagocytic index of the 5 groups of mice stimulated with *Mycobacterium phlei*. The conventional interpretation of the plotted data would include the following 3 points: 1. The untreated 6 month old mice had livers and spleens which were proportionately more efficient than these phagocytic organs of both younger and older mice. 2. Nevertheless, these 6 month old mice responded as the other groups to *Mycobacterium phlei* administration with a comparable increase in "corrected" phagocytic index. 3. No second peak was found and the hypertrophied organs would appear to be less active on a weight basis than the hepatosplenic tissue of untreated mice.

However, we are inclined to question (at

least with regard to mice) the validity of the widely accepted method of correcting the global phagocytic index K to express the efficiency of the liver and spleen on a proportionate weight basis. Biozzi *et al.* (5) proposed the relationship: "corrected" phagocytic index $a = (W/Wls)\ \sqrt[3]{K}$, where W is body weight and Wls is the sum of the liver and spleen weights. Although this formula was derived from a large body of data acquired by studying carbon clearance in the rat, it has failed to muster a logical explanation in the years since its proposal and general adoption.

In our experience with a selected strain of inbred mice, the liver weight, spleen weight and K value of control animals did not vary so widely as indicated by the cited reference. Fig. 3 shows the range of the K *vs* W/Wls points obtained from 59 untreated mice and also presents the curve for untreated rats which was published by Biozzi *et al.*(5). It is evident that our values do not follow the curve. For rats, K values are spread from 0.010 to 0.160 whereas K figures obtained on our mice ranged from 0.007 to 0.018. For the mice, the arithmetic mean for K was 0.010 with a standard deviation of 0.002 and the mean W/Wls value was 15.77 with a standard deviation of 1.32. Therefore, the use of the equation for a is unnecessary for control mice. Furthermore, the cube root relationship which corrects the data of Biozzi *et al.* to 10% of a constant a value for untreated rats does not materially rectify our mouse data. It is our opinion that untreated animals exhibiting wide variations in liver and spleen size are poorly suited to RES investigations. Whenever possible, such animals should be replaced by a more uniform strain of the species.

There is a need, however, for some system of rating the phagocytic efficiency of the enlarged livers and spleens which generally develop from administration of RES stimulants. We employ a simple relationship which receives experimental support from the results of the present study. The formula is $K_{LS} = 10^3\ K \frac{LS_C}{LS_E}$, where K_{LS} represents the relative phagocytic activity of liver and spleen, K is the usual kinetically derived global phagocytic index, LS_C is the average percent body weight of liver and spleen of a control group, and LS_E represents average percent body weight of liver and spleen of an experimental group. The factor 10^3 was added to produce whole numbers rather than decimals to the third place.

Fig. 4 represents the results of our study in terms of K_{LS} and stands in contrast to the curves obtained by plotting the a values derived from the same experimental data (Fig. 2). In our view, Fig. 4 better represents the facts because it shows 2 peaks and the second peak is considerably smaller than the first. As an illustration, one may consider the results from 12 month old mice. Following injection of *Mycobacterium phlei,* the K values at peaks 1 and 2 were 0.056 and 0.058, respectively, and corresponding average percent body weight values of liver and spleen were 6.35% and 11.62%. Since the K values were more than 5 times greater than shown by untreated control mice and the phagocytic organs never reached twice normal weight, one expects both corrected phagocytic indices to be considerably above normal. However, while a for control mice was 4.0, the a figures were 6.0 for K peak 1 and only 3.5 for K peak 2. Using the recommended equation, one obtains K_{LS} values of 10 for the control group, 48 at peak 1 and 27 at peak 2. It is our consideration that K_{LS} is a more meaningful and accurate expression of the experimental facts than a.

Summary. A single intravenous injection of *Mycobacterium phlei* Halpern into mice (1 to 12 months old) elicited a bimodal stimulation of global phagocytic activity. The liver and spleen became enlarged, but returned to normal size after about 2 months. A simple formula was developed for rating the efficiency of the phagocytic organs of mice by taking into account the hepatosplenomegaly accompanying RES stimulation.

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B_{12} Absorption by Monkey Intestine.* (28130)

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Johnson and his collaborators(1), using an isolated loop of jejunum *in vivo*, showed that human gastric juice stimulates B_{12} uptake in the monkey. Uptake in the absence of intrinsic factor (IF) was negligible. The present study extends the comparison of B_{12} absorption in the monkey and the human by establishing the site of maximal intestinal uptake of the vitamin and by characterizing the species specificity of IF activity for the rhesus monkey.

Materials and methods. Sacs of intestine (about 1 g each) were prepared from the small intestine of the monkey according to the method of Wilson and Wiseman(2) and incubated in Erlenmeyer flasks containing 10 ml of Krebs-Henseleit bicarbonate-saline(3) with 200 mg% glucose. Co^{57}-labeled Vit. B_{12} was added to the mucosal compartment at a concentration of 1 mμg/ml medium, with or without a preparation to be tested for IF activity. About 2 ml of bicarbonate-saline containing glucose but no B_{12} were placed inside the sac (serosal side). The flasks were gassed with 95% O_2-5% CO_2 and incubated for 1 hr at 37°C with shaking. Sacs were then carefully washed with 0.15 M NaCl, drained of their serosal contents, blotted and weighed. The radioactivity of the tissue was counted in a well type scintillation counter. The B_{12} taken up was expressed in mμg/g of tissue.

Results and discussion. Intrinsic factor had no effect on B_{12} uptake in the duodenum and upper jejunum, but produced increasing stimulation as the ileo-caecal valve was approached (Fig. 1). In the low ileum IF produced an average stimulation of 70-fold of B_{12} uptake. In the absence of IF very little of the vitamin appeared in the tissue at any level of the small intestine. The site of maximal B_{12} uptake in the monkey is similar to that observed in man(4-9).

The species specificities for IF activity in the monkey and man are also found to be identical. Intrinsic factor from human(10), hog(11,12), monkey(13) and rat(14,15)

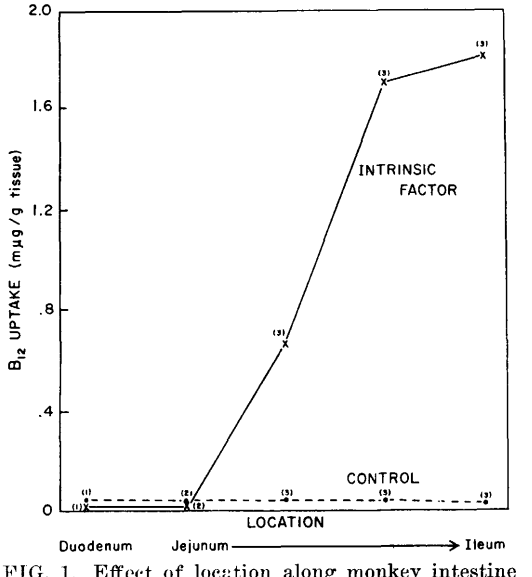

FIG. 1. Effect of location along monkey intestine on B_{12} uptake with and without intrinsic factor. The source of intrinsic factor was a whole homogenate of rat gastric mucosa (2 mg/ml). Numbers in parentheses indicate number of animals used.

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