molysin-forming cell response to endotoxin appears also relevant.

Studies on the effects of these and additional analogs upon antibody formation and priming and their implications for the mechanisms of the immune response will be reported separately.

Summary. The immunosuppressive effect of chloramphenicol *in vivo* has been demonstrated in mice at low dose in short-term experiments by application of the technique of localized hemolysis in agar. The antibiotic suppressed the increase in numbers of hemolysin-forming spleen cells found 42 hr after immunization with sheep red blood cells or treatment with endotoxin. Cetophenicol, an analog of chloramphenicol having the same antibacterial spectrum and ability to inhibit *de novo* protein synthesis, also suppressed the response to the specific antigen, sheep erythrocytes. In contrast, however, cetophenicol enhanced the proliferation of hemolysin-forming cells induced by endotoxin. These opposite influences disappeared when the two antibiotics were injected into the same endotoxin-stimulated mice. Neither analog by itself modified the normal background of antibody-forming cells found in unstimulated mice. The implications of these findings for the modes of action of the analogs and for the influence of endotoxin are discussed.

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Variation in Intestinal Transport of L-Valine in Relation to Age* (33428)

MAY NING, SHELDON REISER, AND PHILIP A. CHRISTIANSEN
(Introduced by D. M. Gibson)

Departments of Medicine and Biochemistry, Indiana University School of Medicine and Veterans Administration Hospital, Indianapolis, Indiana 46202

The active transport of amino acids by the small intestine has been demonstrated both in vivo (1–3) and *in vitro* (4–6). Although the general age and/or weight range of the animals used in these studies is stipulated, no information is available on the effect of the age or weight of the animal on amino acid transport. It has been reported that the muscle/plasma ratio of α-aminoisobutyric acid is decreased with age both in the rat (7) and in man (8). The present paper reports the observation of decreasing intestinal transport of amino acids as typified by L-valine with increasing age in rats.

Methods. Weanling male Wistar rats were used as the experimental animal. The rats

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were fed on a Purina Labena pellet diet and water *ad libitum*. The animals were weighed weekly and the age and weight were recorded.

Four animals were sacrificed at intervals of 4, 8, 12, 18, 24, and 40 weeks. The rats were deprived of food for 24 hr prior to sacrifice. The animals were killed by decapitation and the small intestine was quickly removed and equal lengths were trimmed from the proximal and distal ends. The remaining 60-cm length of mid-intestine was everted and six sacs, each approximately 7 cm in length, were made from each animal. It was previously shown that there is only a very small difference in valine transport as a function of intestinal location in this portion of the intestine (9). Each sac was filled with 1 ml of a Krebs bicarbonate buffer (pH 7.4) (10) containing 20,000–67,000 dpm/ml of uniformly labeled L-valine-^{14}C (Nuclear Chicago Corporation, specific activity 6.9 mCi/mmole) and nonradioactive valine to a final concentration of 0.003 *M*. The sacs were placed in 25-ml Erlenmeyer flasks containing 5 ml of the above medium and incubated at 37° for 60 min in a water bath with shaker oscillating 90–100 times/min. The radioactivity of the serosal, mucosal, and tissue homogenates was determined with a gas-flow counter using a micromil window (Nuclear Chicago) and corrected for self absorption by constructing a curve showing the percentage of maximum radioactivity as a function of sample thickness. The radioactivity of each sample was then converted to the apparent radioactivity at zero thickness.

Calculation of data. Valine translocation from the mucosal to the serosal aspect of the intestine was expressed in the three following forms: (i) I/O ratio—the ratio after incubation of the concentration of valine in the inside (serosal) medium to the concentration of valine in the outside (mucosal) medium. (ii) net transport—the increase of valine (μmoles) in the serosal medium after incubation on a 500 mg tissue wet weight basis. (iii) tissue uptake—the amount of valine (μmoles) present in 500 mg tissue wet weight.

Results. Figure 1 shows that the rats

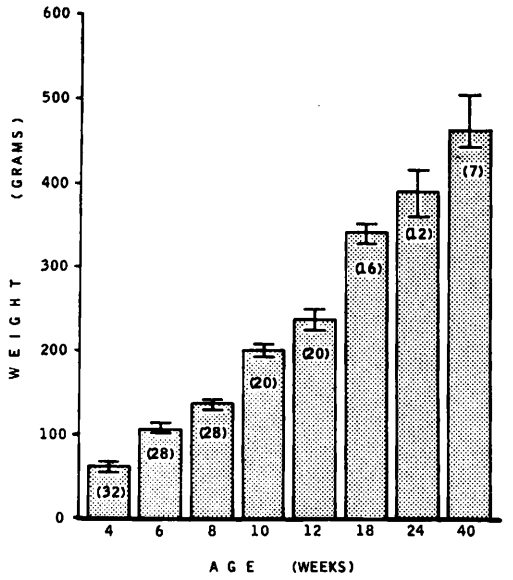

FIG. 1. Each bar represents the mean weights of the rats at the indicated age; in parentheses; number of animals used to obtain the mean and vertical lines are the ranges of the weights.

gained weight rapidly and in a generally linear fashion during the first 18 weeks of age. The average weight gain during this period was 25 g/week. After 18 weeks the rate of increase in weight was markedly reduced. The average weight gain between 18 and 24 weeks was 9 g/week while the weight gain between 24 and 40 weeks was only 4.5 g/week. Our previous studies utilized rats weighing 200–330 g which corresponds to an age of 10–18 weeks.

The I/O ratios were at a maximum during the first 8 weeks of age (Fig. 2). There was no significant difference ($p<0.05$) between the mean ratio at 4 and 8 weeks. After 8 weeks, the ratio fell off precipitously and was only 16% of its 8-week level at 24 weeks. The ratio then leveled off with no significant difference being noted after 24 weeks. The I/O ratio of about 10 found in the 12-week-old rats in the present study agreed well with the I/O ratio previously reported using rats of comparable weights (9). The parameters used to describe valine transport are usually based on the tissue weight to correct for a difference in the number of functional absorptive units per intestinal sac. The I/O ratio is the one

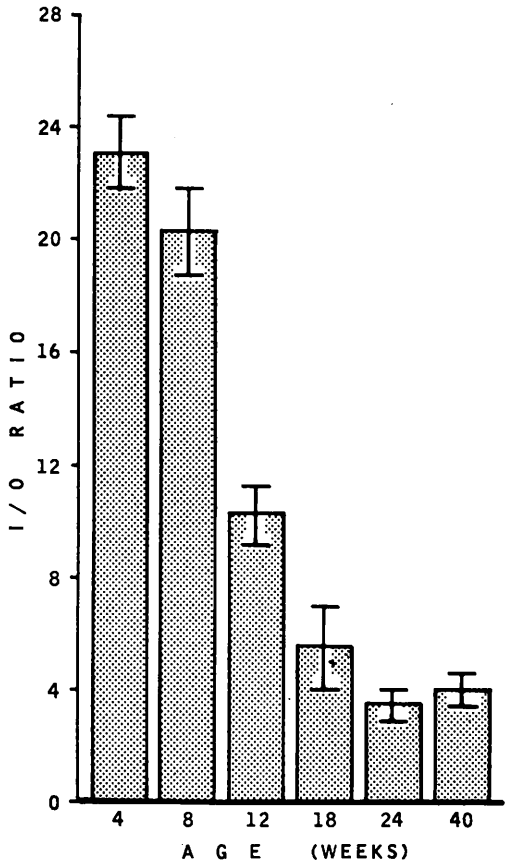

FIG. 2. The I/O ratios from four rats each yielding six sacs were averaged and the mean of the four averages is indicated by the bar. The vertical lines are ± one standard error of the mean (SEM).

parameter not based on tissue weight. Assuming that *in vitro* valine transport is dependent on the number of epithelial cells per sac, the decrease in the I/O ratio with age when there was no corresponding decrease in the number or in the gross morphology of the epithelial cells may be considered evidence for a decrease in the efficiency of *in vitro* transport with increasing age. Extrapolating data from studies in mice, it is probable that the transport decreases long before gross histologic changes or decreased crypt cell activity occurs (11).

The net transport of valine fell with increasing age in a statistically significant manner ($p<0.05$) from 4 to 18 weeks of age (Fig. 3). The most rapid decrease was between 4 and 8 weeks. After 18 weeks there was no significant difference in the transport rates between any consecutive age periods. The decrease in net transport can be related as easily to the increase in weight of the animals as to the decrease in age. Indeed, there was an inverse relationship between net transport and weight as indicated by the relatively constant value obtained when these two parameters are multiplied at any given age. Since net transport is based on a calculated tissue wet weight (9), it is possible that the decrease is due to a relatively large increase in nonabsorptive intestinal tissue. However, the findings using I/O ratio would not be compatible with this possibility. The net

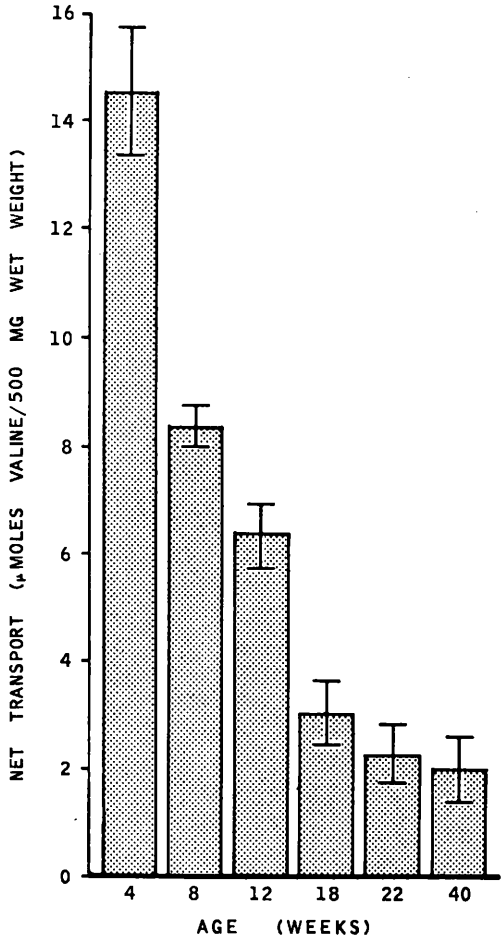

FIG. 3. The net transports from four rats each yielding six sacs were averaged and the mean of the four averages is shown by the bar. The vertical lines are ± one SEM.

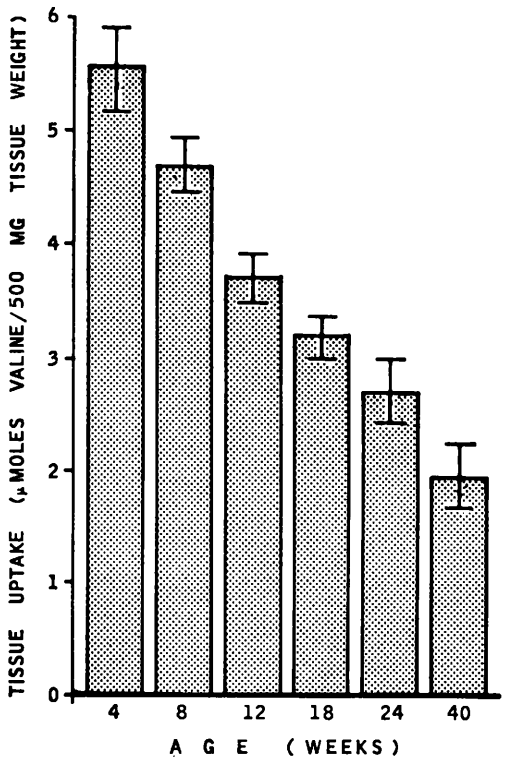

FIG. 4. The tissue uptake values from four rats each yielding six sacs were averaged and the mean of the four averages is shown by the bar. The vertical lines are ± one SEM.

transport of 6.3 μmoles/500 mg of tissue weight found in this study agreed well with the 5.2 μmoles/500 mg of tissue reported previously under the same experimental conditions (9).

Figure 4 shows that the tissue uptake of valine was less sensitive to the age of the rats than were the other transport parameters tested. The decrease in tissue uptake was only significant ($p<0.05$) at the age period between 4 and 12 weeks. The lack of a significant decrease between 12 and 18 weeks differentiates the decrease in tissue uptake from the increase in rat weight which was at a maximum during this age period. The tissue uptake levels obtained in this experiment agreed with that reported previously under comparable experimental conditions. Generally, tissue uptake has not been as sensitive an indicator of a variety of experimental variations as the other parameters (9).

Discussion. In vitro studies on intestinal transport have often been reported using young rats and adult rats. In some experiments no information is given regarding the age or weight of the rat. These animals could have a weight range of from 100 to 400 g. The present study shows that the parameters of valine transport can be decreased from one-half to one-fifth during the age span corresponding to this weight range. It would, therefore, be impossible to compare accurately absolute transport parameters for the same amino acid arrived at by independent studies using rats of widely varying ages. This also implies that reproducible transport data from experiment to experiment in any given study is dependent on the use of rats of a well defined age or weight range. In our previous studies we have employed rats with a relatively wide weight range (9), but with a mean weight of 260 g. The closest age group corresponding to this weight in the present study for which valine transport data were available was the 12-week group. The absolute transport parameters at 12 weeks were found to be in very good agreement with those reported previously (9). This indicates it is possible to obtain reproducible transport data at an age when the transport values are changing rapidly with a minimum of precaution.

Since the magnitude of the transport parameters decreases with age, it is probable that metabolic or competitive inhibitors will produce a smaller decrease in transport in older rats than in younger animals. This may result in a qualitative or quantitative difference in the effect of experimental variables on the transport of the same amino acid in rats of varying age. This may explain some of the conflicting data obtained as to the inhibitory effect of various sugars on the transport of amino acids (12, 13).

It is difficult to dissociate the changes due to age from those due to weight since the age at which the weight increase was most rapid corresponded to the age at which the transport parameters fell off most rapidly. Likewise, the age periods in which the weight gains were relatively small produced a small and generally nonsignificant decrease in the

transport parameters. The decrease in the I/O ratio may be an indication that the age of the animal is most important since this parameter is not based on equal tissue weights.

The mode of regulation by which the rate of amino acid transport is increased at an early age is unknown. There is some evidence that associates pituitary growth hormone with the increased transport of the actively transported, nonmetabolized amino acid α-aminoisobutyric acid (AIB). It has been shown that in the intact rat the steady-state level of AIB in muscle and several other tissues is increased by bovine growth hormone (14). Kostyo (15) and Kostyo and Engle (16) demonstrated an accelerating action of the hormone on the entry of this model amino acid into isolated rat diaphragm. Bovine growth hormone has also been shown to cause a prompt acceleration of the passage of an injected dose of AIB from the plasma into the muscle of the rat (17). These results point to a growth-promoting action of this hormone by an acceleration of amino acid transport and suggests a similar mechanism may be operating in the intestine. However, direct evidence linking growth hormone with an increased intestinal transport of amino acids has not been reported.

Summary. Weanling Wistar strain rats were allowed to age and everted intestinal sacs used to measure various parameters of valine transport at 4, 8, 12, 18, 24, and 40 weeks of age. The rats gained weight at the rate of 25 g/week until 18 weeks of age after which a weight gain of 9 and 4.5 g/week was observed at 24 and 40 weeks, respectively. The ratio of valine in the serosal medium to that in the mucosal medium remained relatively constant during the first 8 weeks after which it fell off precipitously and was only 1/6 its initial level at 40 weeks of age. Net transport of valine fell off in a linear manner between 4 and 18 weeks of age after which it remained at a relatively constant level. At the end of 40 weeks, net transport was 1/7 its initial value. The tissue uptake of valine was reduced 1/3 during the experimental period. The decrease in the transport parameters generally followed the increase in animal weight. The results show the necessity of standardizing the weight or age of the experimental animals used for intestinal transport studies.

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