Multiple agents—carcinogens, X-rays and estrogens—are capable of inducing lymphomas of thymic origin(3), and leukemogenic, filtrable agents have been obtained from lymphomas induced by these different agents.

Radiation-induced lymphomas have been transmitted by cell-free filtrates to newborn mice(9,10), and some recent experiments(4) have demonstrated the "release" of a transmissible leukemogenic agent from irradiated, non-leukemic tissues in C57BL mice shortly after termination of the irradiation.

The isolation of a filtrable agent from estrogen-induced lymphomas in Rf mice has also been reported recently(11).

Attempts to isolate a filtrable agent from chemically-induced lymphomas in mice have been unsuccessful except for the report by Toth(12), who describes the isolation of a leukemogenic agent from DMBA-induced lymphomas in Swiss mice. The present finding of a leukemogenic agent isolated from DMBA-induced lymphomas in C57BL mice coincides with the observation by Toth. (The possibility of some DMBA from the initial treatment being present in the filtrate must be taken into consideration, although this appears unlikely, in view of further successful serial passages having been obtained from the original filtrate.)

The question whether all the different leukemogenic agents induce lymphomas by activation of a latent virus is still debatable. The isolation of a leukemogenic agent from radiation and chemically-induced lymphomas in C57BL mice with a low spontaneous incidence of lymphatic leukemia, encourages the assumption that different leukemogenic agents may act by "activating" a latent virus present in postnatal life in these animals.

Summary. Thymic lymphomas were induced in 60% of C57BL mice fed with 7,12-dimethylbenz(a)anthracene dissolved in polyethylene glycol 400, the mean latent period from the start of the DMBA feedings being 176 days. Three series of cell-free filtrates prepared from the DMBA-induced lymphomas were leukemogenic, producing 15-27% lymphomas in the tested mice. Serial cell-free passages of the original filtrate-induced lymphomas revealed a similar leukemogenic activity of 10-30%.

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Influence of a Fat-Enriched Meal on Human Serum (L-Phenylalanine-Sensitive) "Intestinal" Alkaline Phosphatase.* (31828)

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One of the isoenzymes present in the mixture of alkaline phosphatases in serum has been demonstrated to have the properties of alkaline phosphatase of the striated border of the villi of intestinal mucosa(1-5). This intestinal alkaline phosphatase isoen-

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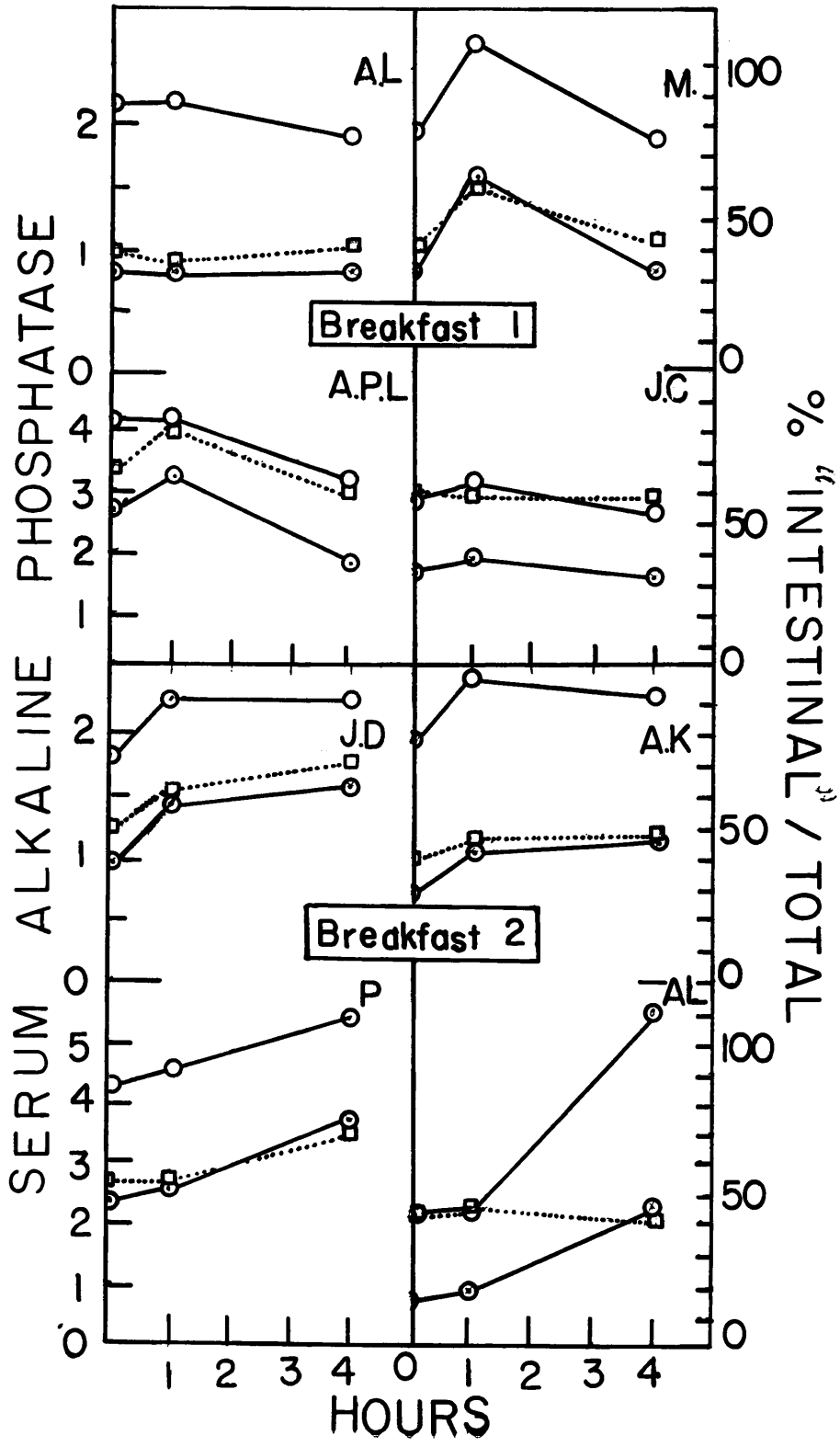
A.L
M.
A.P.L
J.C
J.D
A.K
P
AL
Breakfast 1
Breakfast 2
SERUM ALKALINE PHOSPHATASE
% INTESTINAL / TOTAL
HOURS
0 1 2 3 4 0 1 2 3 4
0 1 2
0 1 2 3 4
0 1 2 3 4 5
0 50 100

FIG. 1. Serum alkaline phosphatase values following ingestion of breakfasts 1 and 2. Total alkaline phosphatase, —O—O—; "intestinal", —⊙—⊙—; and percentage of intestinal to total, ...□...□....

zyme is inhibited by L-phenylalanine and not by D-phenylalanine at 0.005 M concentration in the digest. The intestinal isoenzyme contributes between 20-45% of the total circulating alkaline phosphatase in normal subjects and close to 90% in the hyperphosphatasemia of several patients with cirrhosis(3,4). Both normal individuals and cirrhotics with blood types B and O exhibited a significantly higher concentration of intestinal isoenzyme than do individuals with blood type A(6).

In rats, a fat-enriched diet has long been known to result in an increase in the alkaline phosphatase activity of the intestinal mucosa and the serum(7,8).

It is logical to ask whether the serum L-phenylalanine-sensitive "intestinal" alkaline phosphatase would be raised in a human following the ingestion of a fat-enriched diet. We now report the findings resulting from an experiment designed to answer this question.

Methods. Subjects did not take nourishment or drink after 9 P.M. of the evening before the test day. Two breakfasts were ingested; breakfast #1 consisted of orange juice, 2 eggs, toast, butter and coffee (491 calories), containing fat, carbohydrate and protein in the following amounts, 27.3 g, 48.2 g, 13.0 g. Breakfast #2 was a butter-enriched breakfast of bread, butter, sugar, grape jelly and orange juice (1002 calories). The distribution in grams, of fat, carbohydrate, and protein were, respectively, 69.6, 87.5 and 6.5. The meal was ingested within 15 minutes after a blood specimen was drawn at 8 A.M. At 1 and 4 hours later venous blood specimens were taken. The sera were separated and were submitted to the lab, coded by number only. Measurements were made of the L-phenylalanine-sensitive and total alkaline phosphatases according to Fishman *et al*(4), and were expressed in Shinowara units per 100 ml of serum. In this paper, L-phenylalanine-sensitive and "intestinal" alkaline phosphatase refer to the same moiety described earlier(4).

Results and discussion. The data for 8 normal subjects in this experiment appear in Fig. 1. It may be seen that 2 out of the 4 subjects ingesting breakfast #1 showed a transient increase of "intestinal" alkaline phosphatase at 1 hour. This was also reflected in the percentage of intestinal to total curve. In the 4 subjects ingesting breakfast #2, with the higher fat content, by 4 hours all had shown an increase in the intestinal component and in total alkaline phosphatase compared to the fasting level. However, the rate of appearance was rapid (1 hour) in J. D. and A. K. but somewhat delayed with subjects P. and A.L.

It is clear that whether or not following breakfast an increase in the serum of the intestinal component of alkaline phosphatase occurs, and whether or not the rate of such an increase is rapid and prolonged, both depend greatly on the individual and the fat content of the diet.

In humans, alkaline phosphatase of intestinal origin (as identified by gel electrophoresis) has been found by Keiding(9) to increase in the intestinal lumen and in the thoracic lymph stream following the ingestion of a fat meal. It seems reasonable, therefore, to explain the elevated total alkaline phosphatase by the increment in intestinal alkaline phosphatase especially in subjects on breakfast #2 and to attribute this finding to the absorption of fat from the intestine. The individual differences in pattern may be related possibly to genetic factors as suggested by the ABO blood group studies(6). A discussion has appeared earlier(5) on the role of alkaline phosphatase in intestinal absorption.

Summary. In 2 out of 4 subjects ingesting breakfast containing 27.3 g of fat, a transient increase of the L-phenylalanine-sensitive "intestinal" alkaline phosphatase isoenzyme occurred in the serum. In 4 out of 4 subjects ingesting breakfast with higher fat content (69.6 g) the rise appeared and persisted for 4 hours. Individuals differed greatly in the pattern of serum enzyme partition

which were exhibited. Fat absorption is linked with the later appearance in the serum of the L-phenylalanine-sensitive isoenzyme of alkaline phosphatase.

NOTE ADDED IN PROOF: A recent interesting report by Langman *et al* (Nature, 1966, v212, 41) describes the relevance of the genetic background to the extent of fat-induced increases in serum "intestinal" alkaline phosphatase.

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Effect of Anthocyanin Pigments on Certain Enzymes.* (31829)

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Anthocyanins have been reported to be antibacterial against *Escherichia coli*(1,2) and *Salmonella typhosa*(3) but others have observed no effect on *Staphylococcus aureus*(4) or on pathogens(5). Charles and Niviere(6) found the anthocyanin pigments of red wine to be antiseptic and to inhibit fermentation. Lucia(7), in a review of the properties of wine, cited several reports on the antibacterial properties of wine or wine components. Pratt *et al*(8), Powers *et al*(9) and Hamdy *et al*(10) in separate studies, investigated the influence of anthocyanin pigments on *E. coli, S. aureus* and *Lactobacillus casei* and found that inhibition or stimulation of growth varied with the anthocyanin pigment and organism tested. Powers(11) reported on the effect of 24 anthocyanins, leucoanthocyanins and phenolic acids on the respiration and reproduction of 10 different bacteria.

Although it is well established that anthocyanin pigments do influence the growth of bacteria, the exact mechanism of influence is not understood. Leucoanthocyanin and anthocyanin compounds have been demonstrated to possess biologic activity toward enzymes and plant processes. Steward and Shantz(12) observed that cocoanut milk and horse chestnuts each contained a leucoanthocyanin which stimulated cell division of plants. Hulme and Jones(13) reported that anthocyanins and anthocyanidins inhibited succinoxidase and that leucoanthocyanins inhibited both succinoxidase and malic acid dehydrogenase.

From the work of Somaatmadja *et al*(14) it was established that the inhibitory action of anthocyanin pigments could be explained by their chelation of magnesium ions. It was postulated that the inhibitory action of anthocyanin pigments would affect certain metabolic enzymes in the bacteria. In order to determine the exact site of inhibition a systematic investigation of the enzymes of metabolism was begun. The following were selected because they represented 4 different classes of enzymes:

1. alpha glucan phosphorylase—phosphorylases; 2. glycerol dehydrogenase—dehydrogenases; 3. malate dehydrogenase—dehydrogenases; 4. hexokinase—kinases; 5. glutamic acid decarboxylase—decarboxylases.

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