since the thin cortex and the optic lobes showed no gross lesions. Activation in this study served to demonstrate to us, that the tracings were in fact electroencephalograms and not artifacts.

The prolonged latent period for activation by pentylenetetrazol in the encephalomalacia chicks suggests the importance of midbrain structures and the reticular activating system in induction of convulsions. Further investigation is required.

The reported technics are useful in the study of certain vitamin deficiency states affecting the central nervous system, particularly since such states are readily produced in pigeons and chickens.

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Received June 4, 1962. P.S.E.B.M., 1962, v111.

Dietary Fatty Acids and Plasma Alkaline Phosphatase After Bile Duct Ligation.* (27808)

OTFRID BUTENANDT† (Introduced by Charles Huggins)
The Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Ill.

It is known that the activity of the nonspecific alkaline phosphomonoesterase in plasma of experimental animals increases after bile duct ligation. Large increases have been described in dogs(1,2); smaller increases were observed in the cat(3) and rat (4). It will be shown here that the rise in alkaline phosphatase in the plasma of rat following bile duct ligation is contingent on the presence of unsaturated fatty acids in the diet.

In normal rats the level of the nonspecific alkaline phosphatase in the plasma depends to a considerable extent on the diet: after starvation or after feeding a fat-free diet the activity of the enzyme is low(5). Adding fat to the diet increases the activity of the alkaline phosphatase(6). Weil and Russell(5) found that among the lipid components of the food only unsaturated fatty acids are able to increase the enzyme activity.

Since it would appear that the increased level of the nonspecific alkaline phosphatase in plasma after bile duct ligation is of hepatic origin, the question arises if there is also some relation of the level of plasma alkaline phosphatase to the dietary fatty acids after ligation of the common bile duct.

Methods. The experimental animals were albino rats of Sprague-Dawley strain and dogs. Blood was drawn in rats by cardiac puncture, in dogs from the femoral vein. Ligation of the common bile duct proximal to the duodenum was done under ether anesthesia.

Alkaline phosphatase in plasma: Blood (0.1 ml) obtained by cardiac puncture was diluted with 0.9 ml of 0.15M sodium chloride, centrifuged and the supernatant removed for analysis. The method for determination of the enzyme and the reaction mixture have been described earlier by Huggins and Morii

* This work was aided by grants from Am. Cancer Soc. and Jane Coffin Childs Memorial Fund for Med. Research.

† William Scott Bond Fellow.

(7). One unit is defined as the activity of the enzyme which liberates 1 μM of *p*-nitrophenol at pH 9.3 in 1 hour at 37°C. The units are expressed in terms of 1 ml of plasma.

Bilirubin in plasma: Direct and total bilirubin was determined in diluted plasma using the diazo reaction(8). The reaction mixture consisted of: distilled water, 1.2 ml; diazo reagent, 1.6 ml; plasma supernatant, 0.2 ml. The blank contained 0.4M HCl, 1.6 ml, in place of diazo reagent. The determinations were made with a Beckman model DU spectrophotometer at 560 mμ and compared with a standard bilirubin solution.

Diet. The basal diet‡ consisted of dextrin, 56.3%; cellulose, 6%; egg albumen, 24.6%; salt mixture, 4.8%; vitamin mixture, 1.2%. The diet was supplemented with fatty acid, 7.1%, either stearic acid or oleic acid. The diet was then diluted 1:1 with water.

Results. Bile duct ligation and tube-feeding. In a typical experiment, repeated many times, 6 rats were starved overnight, blood was drawn before operation and the common bile duct was ligated. On Day 1, 3 rats were fed 2 meals of stearic acid diet (10 ml) and on subsequent days 3 meals were provided. The same diet was fed to an additional 3 rats with an additional supplement, twice daily, of oleic acid, 1 ml. All food was given by gastric intubation.

After a 16-hr starvation, the plasma alkaline phosphatase was 4 $\pm$ 1.2 units. In rats fed a diet supplemented with oleic acid there was a progressive increase in activity of plasma alkaline phosphatase for 14 days after bile duct ligation; maximum levels were 11.8 $\pm$ 3.9 units. It is remarkable that there was no elevation of alkaline phosphatase after bile duct ligation in rats fed a diet devoid of unsaturated fats; the levels were 4.3 $\pm$ 1.3 units.

Bile duct ligation and diets fed ad libitum. Thirty rats were starved overnight, blood was drawn and the common duct was ligated. The animals were then divided into groups and fed *ad libitum*; 15 rats were fed the stearic acid diet and 15 rats the diet containing oleic acid. In addition 6 control rats, without ligation of the common duct, were maintained on each diet.

In rats with ligation of the bile duct which were fed stearic acid diet, the plasma alkaline phosphatase remained at 4 $\pm$ 1.4 units for 50 days (Fig. 1). The control animals without ligation of the bile duct had an enzyme activity of 3.5 $\pm$ 1.2 units in plasma.

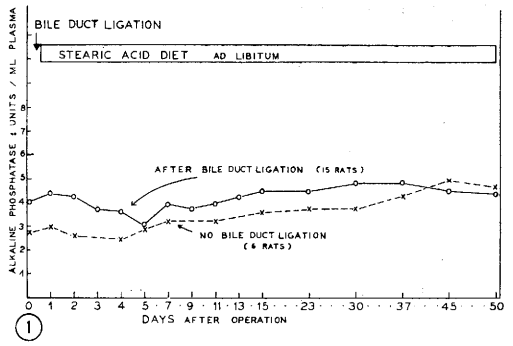
After bile duct ligation in rats fed a diet containing oleic acid there was a progressive increase of alkaline phosphatase activity (Fig. 2). Between days 14-63, the plasma alkaline phosphatase was 11.2 $\pm$ 3.5 units. In control rats without bile duct ligation, fed the oleic acid diet, plasma alkaline phosphatase level on Day 1 was 9.7 $\pm$ 1.1 units; in the next few days the alkaline phosphatase decreased to 6.7 $\pm$ 1.5 units where it remained for 48 days (Fig. 2). After Day 48, in some rats (only those consuming oleic acid diet) very high values of plasma alkaline phosphatase ranging to 26 units were found after bile duct ligation.

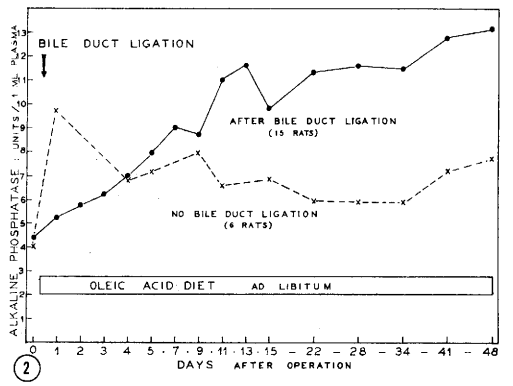
Plasma bilirubin after bile duct ligation. The bilirubin content of plasma of normal rats is very low, 0.01 $\pm$ 0.0015 mg. The plasma bilirubin 48 hr after ligation of the common duct was 0.11 $\pm$ 0.01 mg, regardless of the composition of the diet, indicating complete or a very high grade biliary obstruction.

Bile duct ligation in the dog. Four dogs were fed *ad libitum* the same synthetic diets which had been provided to the rats for 3-6 days prior to ligation of the common duct; 2 of these received stearic acid diet and 2 were fed oleic acid diet. The dogs lost no weight while on the diets. The activity of alkaline phosphatase in all 4 dogs was low during this time (0.81 $\pm$ 0.13 unit).

Following ligation of the common duct the alkaline phosphatase level of the plasma promptly rose to high values (Fig. 3) regardless of presence or absence of unsaturated fatty acids in the food. In all of the dogs

‡ The dietary components were obtained: dextrin and cellulose (Alphacel), Nutritional Biochemicals Corp., Cleveland, Ohio; albumen, Armour Creameries, Chicago, Ill.; fatty acids, Mann Research Laboratories, New York. The fatty acid content of the commercial products was determined by gas chromatography.





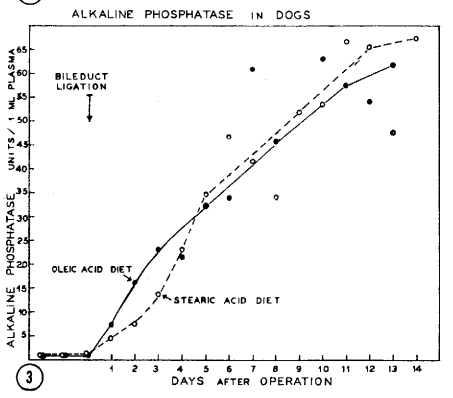


FIG. 1. No elevation of plasma alkaline phosphatase occurred in 50 days after ligation of common bile duct in rats fed stearic acid diet.

FIG. 2. Elevation of plasma alkaline phosphatase after ligation of common bile duct in rats fed oleic acid diet.

FIG. 3. Lack of influence of unsaturated fatty acid in diet on plasma alkaline phosphatase of dogs after ligation of common bile duct.

the bilirubin content of plasma increased after bile duct ligation to high values, 0.86 ± 0.16 mg/ml.

Discussion. In early work the increase of alkaline phosphatase of plasma after bile duct ligation was attributed to retention of the enzyme normally excreted in bile. But Gutman *et al.*(9) found that alkaline phosphatase increases also after ligation of a single hepatic duct in the dog demonstrating in this condition an increased hepatic production of alkaline phosphatase that is retained in plasma. Flock and Bollman(10) found high values of alkaline phosphatase in plasma of rats after bile duct ligation and low values in rats with fistula of the bile duct. These experiments demonstrate that absence of bile in the intestine is not the cause of the increased plasma alkaline phosphatase after bile duct ligation.

The present work shows that, in the rat, diet of unsaturated fatty acids is essential for the increase in plasma of alkaline phosphatase (but not of bilirubin) after bile duct ligation. It has been found(11,12) that *ca.* 80% of both saturated and unsaturated fatty acids are absorbed from the intestine. The mechanism explaining why unsaturated fatty acids are essential for the pronounced rise in alkaline phosphatase is obscure. It would appear that in the absence of unsaturated fatty acids the liver of the rat is unable to produce large quantities of alkaline phosphatase after ligation of the common bile duct.

Summary. Unsaturated fatty acids in the diet exert a profound influence on the level of alkaline phosphatase in the plasma of rats with biliary obstruction as well as in normal rats. Following ligation of the common bile duct, alkaline phosphatase was increased about 3-fold in rats fed a diet containing oleic acid. No rise of alkaline phosphatase occurred after biliary obstruction in rats fed the same diet containing no unsaturated fatty acids. Rise in plasma bilirubin occurred after ligation of the common duct regardless of the content or composition of fatty acids in the diet. In the dog with ligation of the bile duct no relation was detected between unsaturated fatty acids in the diet and the increased (about 60-fold) alkaline phosphatase of the plasma; the enzyme rose to very high and nearly similar levels in dogs fed diets with or without unsaturated fatty acids after ligation of the common bile duct.

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Received June 11, 1962. P.S.E.B.M., 1962, v111.

Influence of Surface Charge of Phospholipids on their Clot-Promoting Activity.* (27809)

DEMETRIOS PAPAHADJOPOULOS, CECIL HOUGIE† AND DONALD J. HANAHAN
Departments of Biochemistry and Pathology, University of Washington, Seattle

The identity of the phospholipid participating in blood coagulation has long been the subject of controversy. It has been maintained that phosphatidyl ethanolamine (PE) is the active phospholipid(1,2), while it has also been indicated that phosphatidyl serine (PS) is the active constituent and that its activity could be potentiated by phosphatidyl choline (PC) or other lipids(3,4). Other specific factors have been cited as essential components for activity. For example, Rouser, *et al.*(5) emphasized the importance of unsaturation of the fatty acids and the presence of a free amino group in the phospholipid. Rapport(6) and Theriault, *et al.* (7) reported that potentiation of the activity of PS by PC is greatly increased if the lipids are first mixed in organic solvents and then emulsified. Wallach, *et al.*(2) showed that the pH of the emulsifying buffer was an important factor for optimal activity of the phospholipid. These investigators observed a gradual rise in activity of PE emulsions between pH 5 to 8, then a rapid increase between pH 8 to 10. This change in activity was attributed to the formation of smaller particles due to electrostatic interactions. Recently, Bangham(8) demonstrated that mixed emulsions containing varying amounts of PE plus PC and dicetyl phosphoric acid plus PC showed increasing activity with increasing surface charge.

Certain of these observations showing an increase of activity by mixing 2 phospholipids, or by changing the pH, were confirmed in this laboratory. It was then felt necessary to investigate further the possible influence of physical factors on formation of the prothrombin activator. The experiments reported here include a study of the "thromboplastic" activity of a series of mixed phospholipid emulsions derived from beef brain and egg yolk, and the influence of the surface charge of the lipid particles on this activity.

Materials and methods. Lipid fractions. PS and PE were isolated from beef brain as follows: Brains received within half hour after slaughter were placed immediately in dry ice. Within one hour, the brains were extracted with C:M (chloroform:methanol) 2:1 (v/v) and total lipid extract obtained as described by Rouser *et al.*(9). Most of the steps were performed under a stream of nitrogen. The final extract was dissolved in chloroform and chromatographed immediately on a silicic acid column (2g silicic acid for each mg phosphorus). The neutral lipids were eluted with C:M 9:1, and subsequent use of

* This investigation was supported in part by grants from Life Insurance Medical Research Fund, and from the National Heart Institute, U.S.P.H.S.

† Established Investigator of Am. Heart Assn.