Discussion. Ethionine used under the conditions of the present experiment is a hepatotoxic agent known to produce several metabolic alterations of liver function. It is apparent that it also interferes with ammonia metabolism.

Meat was used in this study as a test of the ability of the liver to handle this nitrogen rich meal. Rats pretreated for 6 weeks with a diet containing ethionine reacted to the test meal by an abnormal increase of blood ammonia. Addition of methionine to the ethionine diet produced a marked improvement in the reaction of rats to the test meal.

It is concluded that a diet containing ethionine may result in abnormal ammonia metabolism in rats. Methionine, which is known to prevent most of the effects of ethionine, probably has a protective action against the effect of ethionine on ammonia metabolism, under the experimental conditions described here.

Summary. Postprandial blood ammonia was significantly higher in rats fed the diet containing ethionine than in the animals given the normal basal diet. Addition of methionine to the ethionine diet had a protective action against the effect of ethionine on ammonia metabolism. Experimental liver damage by ethionine is considered a cause of abnormal ammonia metabolism.

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Received September 2, 1964. P.S.E.B.M., 1965, v118.

Site of Origin of Increased Fibrinolytic Activity During Venous Occlusion.* (29884)

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Fibrinolytic activity of the blood is increased in conditions such as strong physical or emotional stress(1) and by venous occlusion(2). The site of origin of increased fibrinolytic activity of the blood is not known. Mole demonstrated that, after sudden death, blood in limb veins exhibited greater fibrinolytic activity than blood collected from the heart. He postulated that vascular endothelium was the source of increased fibrinolytic activity after sudden death(3). Fearnley reported that the fibrinolytic activity of citrated plasma was increased by placing it for 2 minutes in an isolated segment of an exposed vein. This author concluded that the wall of large veins adds fibrinolytic activity to the blood(4).

In the present investigation venous occlusion was used as a method to gain additional information on the site of origin of increased fibrinolytic activity.

Materials and methods. The subjects used in these experiments were healthy male medical students and staff members, ages 18 to 42. In each experiment a control blood sample was collected from an antecubital vein

* Supported by grants from U.S. Public Health Service and Am. Cancer Soc.

TABLE I. Fibrinolytic Activity and Protein Content of Plasma Samples Collected Before and Serially After Venous Occlusion.

Period of occlusion	No. of exp	Before occlusion	Serial samples collected after occlusion 1	2	3	4	5
(min.)		Fibrinolytic activity of euglobulin fraction as mm² of lysis (mean ± S.E.)					
5	5	128 ± 18	182 ± 30	219 ± 39	234† ± 39	231† ± 29	217 ± 30
10	5	151 ± 16	252 ± 31	311† ± 44	342* ± 46	349* ± 48	326† ± 40
		Plasma protein concentration in gram per 100 ml (mean ± S.E.)					
5	5	7.48 ± .20	7.80 ± .22	8.16 ± .30	8.24† ± .30	8.60* ± .22	8.72* ± .17
10	5	7.76 ± .17	9.08 ± .15	9.84* ± .09	10.12* ± .13	10.40* ± .13	10.48* ± .20

* Mean significantly different from mean of postocclusion sample 1 at 1% level.
† *Idem.* at 5% level.

without the use of a tourniquet. Then a blood pressure cuff was placed above the elbow of the other arm and the cuff pressure raised to midway between systolic and diastolic pressure. After 5 or 10 minutes of occlusion, a needle attached to a 3-way stopcock was introduced into an antecubital vein of the occluded arm. A series of 5 blood samples was collected rapidly in separate syringes within a period of 60 seconds before releasing the pressure in the cuff. Each sample consisted of 4.5 ml blood mixed with 0.5 ml trisodium citrate solution, 0.129 M. The citrate concentration of the plasma was adjusted to be equal in each sample. Isolation of the euglobulin fraction from plasma, measurement of the fibrinolytic activity of the euglobulin fraction by the standard bovine fibrin plate method, determination of plasma protein concentration, white blood cell counts, and determination of hematocrit were carried out according to methods reported previously (5).

The fibrinolytic activity of euglobulins, measured in mm² of lysis on standard bovine fibrin plates, was converted into units of euglobulin activity using a euglobulin reference curve. Such a curve was obtained by plotting fibrinolytic activity (in mm²) *versus* concentration for a series of dilutions of a highly active, two-fold concentrated, postocclusion euglobulin preparation. A value of 100 units was arbitrarily assigned to the fibrinolytic activity of undiluted postocclusion euglobulins.

To concentrate plasma proteins *in vitro*, use was made of the pervaporation method (6). Five ml of freshly collected citrated normal plasma were placed in a seamless cellulose bag and kept at 4°C for 1 to 6 hours in the air current generated by a fan.

Statistical analysis. For determination of the significance of differences in fibrinolytic activity and protein content of postocclusion samples, the standard error of the difference between any 2 of the means of postinjection samples was calculated as $\sqrt{\frac{2s_e^2}{N}}$ where s_e^2 is the mean square of the error given by the analysis of variance of the data, and N the number of subjects studied. Dunnett's one-sided multiple comparisons test was used to test the means of postocclusion samples 2 to 5 *versus* the mean of postocclusion sample 1(7).

Results. Table 1 represents results of determination of fibrinolytic activity and protein content in a series of rapidly collected plasma samples after 5 or 10 minutes of venous occlusion. The fibrinolytic activity increases from the first through the third or fourth postocclusion samples, then reaches a point of maximal activity, and subsequently declines again. The increase in fibrinolytic activity in postocclusion blood samples is accompanied by an increase in protein concentration of plasma which is directly proportional to the increase in protein content of the euglobulin fraction(5). Consequently, to compare the true increase in fibrinolytic activity in these postocclusion samples, it is necessary to calculate the specific fibrinolytic activity or ratio of units of euglobulin activity per ml to the protein content of the plasma in mg per ml. Fig. 1 demonstrates that there is a progressive increase in specific fibrinolytic activity from postocclusion sam-

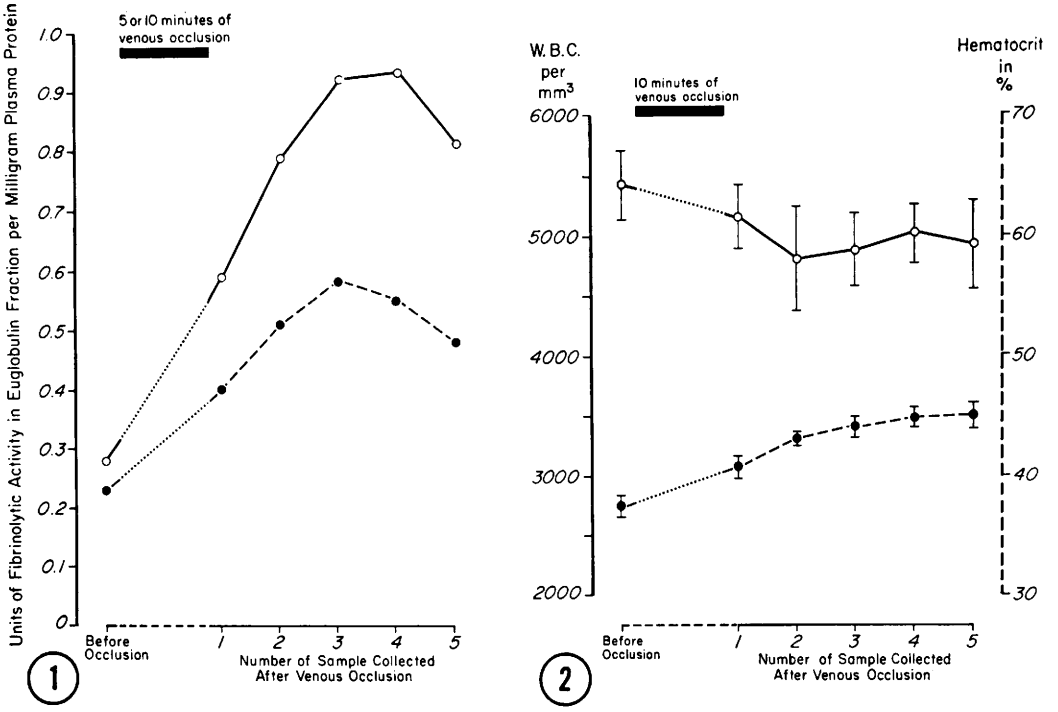

FIG. 1. Fibrinolytic activity of the euglobulin fraction in units per mg plasma protein in samples collected before and serially after venous occlusion. Points represent mean values of 5 experiments with 5 min of venous occlusion (dashed line) and of 5 experiments with 10 min of venous occlusion (solid line). With 5 min of venous occlusion the mean of postocclusion sample 3 is significantly greater than the mean of postocclusion sample 1 ($P < .05$); with 10 min of venous occlusion the means of postocclusion samples 3 and 4 are significantly greater than the mean of postocclusion sample 1 ($P < .05$).

FIG. 2. White blood cell count and hematocrit in blood samples collected before occlusion and serially after 10 min of occlusion. Points represent mean values of 5 single experiments and vertical lines a single standard error of mean.

ple 1 to 3 after both 5 and 10 minutes of venous occlusion.

Control experiments demonstrated that specific fibrinolytic activity remained unaltered when the protein content of plasma was increased by 20 to 150% by the pervaporation method.

The hematocrit exhibited a progressive increase after venous occlusion. Although one would expect the leucocyte count to show an increase of equal magnitude, it decreased (Fig. 2). These data suggest that leucocytes had either migrated out of the vascular bed, adhered to walls of small blood vessels or lysed within the circulation.

Discussion. This study demonstrates that there is a progressive increase in blood fibrinolytic activity from the first through the third or fourth blood sample collected in rapid sequence after a period of 5 or 10 minutes of venous occlusion. The decline in fibrinolytic activity in the fifth sample indicates that the increase in fibrinolytic activity in the earlier samples cannot be explained by the fact that some time elapsed between collection of successive postocclusion samples. It can be assumed that the first postocclusion blood sample consists mainly of blood which was contained in large veins and that subsequent samples drained progressively smaller blood vessels. The progressive increase in fibrinolytic activity in the first 3 or 4 blood samples indicates that the smaller veins and/or the capillary bed are the major site of origin of the increased fibrinolytic activity of the blood.

It is interesting to speculate that the fibrinolytic activity found in association with the endothelial lining of blood vessels(8,9,10) is released into the blood during venous occlu-

sion. Since the concentration in the blood of any substance released from vessel walls must necessarily be a direct function of the ratio of blood vessel surface to blood vessel caliber, one would expect a progressive increase in fibrinolytic activity in serial blood samples collected after venous occlusion.

Aside from a release of fibrinolytic substances from vessel walls, other possible mechanisms must be considered for the production of increased fibrinolytic activity in small vessels during venous occlusion. Destruction of white blood cells could release leucoprotease. Such a mechanism has been suggested by Gotschall(11). The observation that the number of white blood cells is decreased in the postocclusion samples would be compatible with this hypothesis. Another possibility is that contact activation contributes to the increase in blood fibrinolytic activity. Addition of kaolin to plasma *in vitro* enhances greatly plasma fibrinolytic activity, presumably through activation of Hageman factor(12). It is conceivable that venous occlusion could produce alterations in the inner lining of small blood vessels which might cause contact activation of the blood fibrinolytic system. In support of the last hypothesis is the observation that the fibrinolytic response to venous occlusion is smaller in persons with Hageman trait than in normal individuals(13).

Summary. The fibrinolytic activity of serial blood samples, 5 ml each, collected rapidly one after another after 5 or 10 minutes of venous occlusion of the forearm, increases progressively from the first through the third or fourth sample. This observation indicates that small venous tributaries and/or capillaries are a major site for production of increased fibrinolytic activity during venous occlusion.

The author is indebted to Dr. G. D. Penick for reviewing the manuscript, and to Dr. J. Grizzle for assistance in statistical evaluation of the data.

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Received September 2, 1964. P.S.E.B.M., 1965, v118.

Substrate Inhibition of Tetrazolium Salt Reduction in Dark Adapted Retinae.* (29885)

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Enoch(1) reported that the light adapted retina of rat, rabbit, and squirrel monkey reduced more nitroblue tetrazolium chloride (nitroBT) in simple buffered media using succinate as substrate than did the fellow dark adapted retina. The effect was enhanced when the tissue culture medium, TC-199 without phenol red, was used and the effect was reduced if ATP was added to the media. Sickle(2) has reported results which suggest that light stimulation of isolated frog retinae alters metabolism or metabolic pathways.

* This research was supported by funds from Research to Prevent Blindness, Inc., contract DA-49-193-MD-2457 U. S. Army Med. Research and Development Command, and NIH Grant B1979.