results with albino rats indicate that the strain difference is not a matter of whether the rats are albino or pigmented, but it is possible that the beneficial effects of glutamic acid might be specific to the Sherman strain of albino rats. At the present time experiments are being designed to test animals of the Sherman strain. Further experiments will be done in which proline and other compounds metabolically related to glutamic acid will be studied. At this point, however, it must be concluded that there is little evidence for a facilitating effect of excess glutamic acid feeding on the learning ability of the rat.

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Effect of Heparin and Dicoumarol on Sludge Formation

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Since Kniseley published his findings of the sludge phenomenon (*2*) in shock and certain other disease states it has been assumed that this condition was a precursor of thrombosis, as evidenced by the following quotation from a recent editorial in the *Journal of the American Medical Association* (*4*):

> As a result of publication of this report (Kniseley), many practicing physicians have suggested use of heparin or Dicoumarol to prevent the sludging of blood of patients met in their daily practices. Until these observations have been extensively checked by other investigators, introduction of new methods of treatment to combat sludging of blood should be highly experimental.

In the course of vascular occlusion experiments in dogs (*3*), we found that we could produce sludge at will in the smaller vessels distal to an occlusion within a relatively short time. Since our studies included an evaluation of various therapeutic agents in vascular occlusive states, we felt it necessary not only to determine the effects of anticoagulants on thrombus formation, but to investigate their effects on sludge formation as well.

Therefore, an experiment was set up to determine the effects of heparin and Dicoumarol on sludge formation in small vessels following acute main-stem venous occlusions. We used venous occlusions exclusively, since the process was usually more gradual than in the arterial occlusions and could be followed more closely.

All observations were made on mesenteric vessels, using a Kniseley fused quartz rod transillumination apparatus (*1*) employing a constant-temperature tissue bath with variable volume flow. Young, small dogs were used, and intravenous Nembutal anesthesia was employed. A special Lucite tray held the dog's mesentery in a nonstretched position, submersed in constantly circulating mammalian Ringer's solution at body temperature. The microscope was fixed on small vessels. A small precapillary artery and vein running side by side were chosen for observation, the artery measuring from .054 to .144 mm in the various animals and the vein from .090 to .288 mm. The capillaries stemming from such vessels were observed in the same microscopic field.

In over 70 main-stem venous occlusions done on normal dogs we had consistently noticed sludge formation in the small vessels within 10–20 min following occlusion. Six animals in this group, chosen at random, comprised our control series. A second group of 6 animals was used in a preliminary experiment in which heparin was given by intravenous injection into a tongue vein after sludge formation was noted. A third group of 6 animals was heparinized before venous occlusion, while a fourth group of 6 animals was dicoumarolized before venous occlusion.

Group I: Control. The portal vein or superior mesenteric vein was occluded in the usual manner by a rubber-tipped clamp which was allowed to remain in place for about 1 hr before release. As the stream slowed and anoxia progressed, sludge formation was noted in each instance within 10–20 min in the small peripheral mesenteric vessels. As the stream slowed further, thrombus formation was noted in some of the small vessels, especially in the capillaries and venules, usually within 30 min after the appearance of sludge. The groups of agglutinated cells traveled in spurts through the spastic artery, while in the vein they became attached sooner or later to the endothelial lining of the vessel. As a small group of cells became adherent to the side of the vessel, more cells became attached to the mass, and a thrombus formed which eventually obliterated the entire lumen as the stream stopped flowing. In many instances, if the occlusion was released at this stage, flow again resulted as the liquid stream washed the agglutinated particles through. Other small vessels maintained their thrombosis and did not become patent. For the most part, by the time of release (about 1 hr), the flow had either ceased completely or was in the ebbing stage within most small vessels. After release of the occlusion, the vessels not showing renewed motion of the stream were considered thrombosed.

The process of thrombosis, then, in the small vessels under microscopic observation appeared to consist of the following steps: (a) sludge formation, (b) adherence of sludged masses to the endothelial lining of the vessels, (c) the "piling up" of more cells to such an agglutinated mass, and (d) stoppage of flow after complete occlusion of the vessel by an agglutinated mass of cells.

Group II: Heparin administered after the appearance of sludge formation. Using dogs of about 5 kilos, he-

parin[1] was administered in doses ranging from 6 to 10 mg. The heparin was injected into a tongue vein as soon as sludge formation was noted following venous occlusion. In no instance was there any alteration in the nature of the sludge. However, it was noted that the clumps of sludged cells did not become adherent to the vessel wall as easily as in the control group. The sludged clumps built up in size until the vessel became almost occluded, but the mass did not become adherent to the endothelial lining. As a result, such masses rocked to and fro or flowed on in spurts. Occasionally such a mass, upon reaching a bifurcation, would split in two after being halted temporarily. Upon removal of the clamp, such vessels all resumed flow, and no thrombosis was observed.

Group III: Heparin administered before venous occlusion. Heparin was injected intravenously in doses ranging from .5 to 1 cc, depending on the weights of the dogs. This resulted in clotting times ranging from 15 to 28 min. After occlusion of the main vein, sludge formation was noted in each instance. Sludge appeared in from 9 to 35 min following occlusion. The stream slowed down in the usual manner but took considerably longer to stop completely than in the control series. In some instances there was considerable ebbing until the time of release. In the control series, as a rule, the majority of vessels were completely agglutinated within 30 min. In this series there was complete agglutination after 45 min in only one instance. In this case perhaps 25% of the vessels in the microscopic field were involved, the remainder maintaining patency. Except for this one instance, the sludged clumps of cells did not become adherent to the endothelial lining. Upon release of the occlusion, flow resumed in each instance, perhaps much more rapidly than in the control group.

It would thus appear that heparinization in clinically therapeutic dosages has no effect on sludge formation but will aid considerably in the prevention of thrombus formation around groups of sludged cells.

Group IV: Dicoumarol administered before venous occlusion. Six dogs were given adequate doses of Dicoumarol for a period of 3 days before the experiment. The doses ranged from 25 to 50 mg/day, depending upon the weights of the dogs. Prothrombin levels on diluted plasma, determined prior to occlusion, ranged from 15 to 20% of normal. Venous occlusions were made in the usual manner, and the small vessels were observed for sludge formation. Sludge appeared in each instance within 10–30 min. The appearance of the flow after approximately 1 hr was no different from that described in the heparinized animals. Again, the sludged masses did not become adherent to the endothelial lining and seemed to break up easily into smaller masses. In no case in this series was there any thrombus formation. Upon release of the occlusion, the blood within the small vessels resumed rapid flow.

Dicoumarolization therefore had no effect upon sludge formation but, as in the case of heparinization, prevented thrombus formation.

[1] Liquaemin, Roche-Organon, Inc., Nutley, New Jersey; 1 cc = 10 mg.

Our observations indicate that sludged masses of blood cells may serve as a matrix for thrombus formation, provided the other conditions favoring thrombosis are present. When anticoagulants are administered in doses known to be clinically effective, yet safe, thrombosis does not generally occur in the small vessels distal to an occlusion, but such doses do not prevent the formation of sludge.

The administration of anticoagulants prevents thrombus formation in the presence of sludge by preventing the sludged masses of cells from becoming adherent to the endothelial lining of the vessel. Although anticoagulants may cause some diminution in the tenacity of the adherence of the blood cells to one another, sludge formation, as such, is not prevented.

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An Experiment on Human Vitamin B_6 Deprivation[1]

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For some years there have been reports of the successful therapeutic use of pyridoxin hydrochloride in several disorders and disease symptoms, some of which have accompanied a state of malnutrition. Whether in these cases the action of the compound accrued from any nutrient property is difficult to judge, mainly because of the magnitude of dosage. Under such conditions a pharmacological rather than a physiological action of vitamins should be considered, and interpretations of nutritional significance should be weighted accordingly.

The nutritional need of several organisms, including some mammals, for vitamin B_6 compounds has been established, and something is even known of their probable functions in metabolic processes. There is no direct evidence, however, that any of them are nutritionally essential for man, even though the ordinary human dietary supplies them in relative abundance.

Ancel Keys wrote: ". . . the only evidence which we will accept as quantitatively exact on human requirements for B vitamins is that derived from controlled experiments on man. . . . The needs for controlled experimental studies on man are glaring" (*3*).

We are reporting an investigation on the possible human need for vitamin B_6.

An experiment was carried out on an adult male (W. W. H.), who subsisted upon a purified diet and re-

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