*Discussion and Summary.* Although about 20 of the rare elements have received some attention in pharmacology and some of these (thallium, gallium) have distinctive properties, rhenium surprises observers by its relatively low toxicity and general inertness in the body. The lethal dose for rats is of the order 900-1000 mg per kg of body weight. In rats it lacks the hemopoietic stimulus of Cobalt or Germanium, develops visible symptoms only in large doses, and produces only small transitory changes in blood pressure. Symptoms resembling the convulsions produced by strychnine suggest that the spinal cord has a high selectivity for the salts of Re.

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## Partition Studies on the Clot-Aiding and Related Blood Phospholipids.

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The phospholipid *cephalin* is a potent coagulation (thromboplastic) agent[1] and a natural blood constituent. Elucidation of its distribution and physicochemical state is needed to define its rôle in clotting mechanisms.

The coagulation defect in hemophilia is inadequately remedied by cephalin *(in vitro)*. Trypsin (thromboplastic enzyme[2]), however, restores the normal coagulation properties, especially under optimal conditions with respect to the calcium and phospholipid factors.[3] The cephalin content of the blood platelets, including hemophilic, has been investigated previously.[4] With the aid of recently developed micromethods,[5] the following data (Table I) have been

* This research was carried out with the aid of a Sigma Xi grant.

1 Ferguson, J. H., *Am. J. Physiol.*, 1938, **123**, 341.

2 Ferguson, J. H., and Erickson, B. N., *Am. J. Physiol.*, 1939, **126**, 661.

3 Ferguson, J. H., *Am. J. Physiol.*, 1939, **126**, 669.

4 Erickson, B. N., Williams, H. H., Avrin, I., and Lee, P., *J. Clin. Inv.*, 1939, **18**, 81.

5 Erickson, B. N., Avrin, I., Teague, D. M., and Williams, H. H., *J. Biol. Chem.*, 1940, **135**, 671.

TABLE I.
Phospholipid Partition of Plasma in Hemophilia.
(Mg per 100 cc plasma.)

| | Total Phospholipid | Cephalin | Lecithin | Sphingomyelin |
|---|---|---|---|---|
| Normal* | 189 | 55 | 99 | 35 |
| Hemophilia | 131 | 42 | — | — |
| (5 patients) | 165 | 45 | 66 | 55 |
| | 185 | 84 | 29 | 71 |
| | 170 | 49 | 59 | 61 |
| | 114 | 39 | 50 | 25 |

*Values from other studies.[5]

obtained on the partition of the plasma phospholipids (lecithin, cephalin and sphingomyelin) in hemophilia. There is no lack in the amount of the clot-aiding phospholipid cephalin.

*Free and Bound Cephalin.* It has been reported[2] that some prothrombin preparations although containing appreciable amounts of cephalin may be poorly (or not at all) activated by calcium alone. However, when similar amounts of pure cephalin solution are added, a highly active thrombin is formed. It is evident that the rôle of cephalin in this process is specifically dependent upon its physicochemical state or mode of combination. In the state of its natural protein combinations, cephalin does not seem to be available for direct participation in the coagulation rections. An attempt was made, therefore, to distinguish between the free and bound phospholipid in blood plasma.

A variety of methods was tried, including phospholipid analyses of plasma ultrafiltrates, protein-free filtrates, and ethyl ether and petroleum ether extracts of plasma and of plasma dehydrated with plaster of Paris. It was found practicable to recover a "free" phospholipid fraction by extraction with ethyl and petroleum ether after preliminary dehydration of plasma by pervaporation[6] in Visking casing sacs, exposed to warm air for 12 to 24 hours. This procedure was necessary for quantitative recovery (within ±4%) of pure cephalin from simple solution. Complete recovery of phospholipid in the presence of the plasma proteins always required the concomitant action of a denaturating agent, such as ethyl alcohol. The ready resolution and retention of clotting properties of pervaporated plasma (unextracted) support the belief that no significant denaturation and breakdown of the phospholipid-protein complexes occur during the cited procedures. The lipid-extracted dry plasma could be redissolved but had now lost its clotting properties.

The analytical data of Table II show the striking absence of all

[6] Thalhimer, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 230.

but traces (frequently less than the limits of the analytical methods) of "free" phospholipid from most normal and hemophilic plasmas. Positive findings were limited to the lipemias, occurring naturally in some hen plasmas and, as a clinical curiosity, on one human subject, *viz.*, a male child of 8 years,[7] who was known to have had this condition for 4 years without ever evincing any bleeding or clotting anomaly, according to routine clinical tests.

TABLE II.
Free Phospholipid of Citrated Plasma (Including Recovery of Added Cephalin) With and Without Trypsin.

| Original sample | Free phospholipid (mg 100 cc plasma) | Cephalin added (mg 100 cc plasma) | Trypsin added (mg cc plasma) | Free phospholipid (mg 100 cc plasma) |
|---|---|---|---|---|
| Normal | 0-0.2 | — | — | 0-0.2 |
| Human | | 15 | — | 0.2-0.4 |
| Plasma | | 30 | — | 1.4-5.7 |
| | | 30 | 0.5 | 0.4 |
| | | 30 | 2.0 | 2.9 |
| | | 50 | — | 0.4-0.8 |
| | | 50 | 2.0 | 1.1-1.4 |
| | | 100 | — | 0.5-6.7 |
| | | 100 | 2.0 | 2.6 |
| Hemophilic | 0-0.4 | — | — | 0-0.4 |
| Plasma | | — | 0.1 | 0.4 |
| | | 30 | — | 0.6-1.1 |
| | | 30 | 0.5 | 0.5 |
| | | 30 | 2.0 | 7.7 |
| | | 85 | — | 2.3 |
| | | 85 | 2.0 | 2.5-2.7 |
| | | 100 | — | 1.1-1.9 |
| | | 100 | 2.0 | 10.0 |
| Dog Plasma | 0-0.8 | — | — | 0-0.8 |
| boiled | 0.3-0.5 | — | — | 0.3-0.5 |
| | | — | 0.1 | 0 |
| | | — | 0.5 | 0.2 |
| | | — | 2.0 | 0.3 |
| Human Lipemic | 31 | — | — | 31. |
| Plasma | 31 | — | 2.0 | 42. |
| | 87* | — | — | 87.* |
| Hen Plasma | 0.4-188† | — | — | 0.4-188† |
| | 0.4 | — | 3.0 | 0 |
| | 0.4 | 55 | — | 7.0 |
| | 0.4 | 55 | 3.0 | 5.4 |
| | 70. | 55 | — | 90. |
| | 188. | 193 | — | 171. |
| | 188. | 193 | 4.0 | 346. |

*12 mg of this consisted of cephalin.

†The amount of free phospholipid was related to the degree of the lipemia; of the 188 mg, 161 mg consisted of cephalin.

[7] Bernstein, S. S., Williams, H. H., Hummel, F. C., Shepherd, M. L., and Erickson, B. N., *J. Pediat.*, 1939, **14**, 570.

On the addition of free cephalin to non-lipemic plasmas, including hemophilic, very small amounts could be recovered subsequently in the "free" form; nearly all of the phospholipid appeared to have been bound to the proteins.

The addition of crystalline trypsin,† in an effort to free the plasma phospholipid from its protein combinations, yielded variable results. There is no general confirmation of the preliminary data previously reported (on prothrombin solutions)[2] as suggestive of the ability of the proteolytic enzyme to liberate cephalin. Even with added cephalin or lipemic plasma, it was not possible to conclude whether the presence of trypsin modifies the proportion of free to bound cephalin.

*Free and Bound Phospholipid in Platelet Lysates.* Hemophilic platelets are not lacking in essential phospholipids,[4] but platelet lysis is delayed in hemophilia.[8] The question of platelet lysis in relation to the liberation of phospholipid was investigated with the foregoing technic for free and bound phospholipid.

Platelets were recovered from citrated dog plasma by differential centrifugation to free them from plasma and red cells. A few leucocytes were doubtlessly included in the washed platelet residues. The platelet suspension was lysed with N/100 calcium chloride and the lysate removed after prolonged high-speed centrifugation. The residue was boiled in water and recentrifuged. In a first experiment the lysates were completely free from analyzable phospholipid. In a second experiment mere traces, *viz.*, 0.01 mg of "free" and 0.04 mg of "bound" phospholipid were found in the total lysate from the platelets obtained from 500 cc of dog blood. Additional traces were obtained from the extract of the boiled platelet residues. Both solutions, especially the latter, were fairly strongly thromboplastic in clotting tests on dog plasma.

*The Phospholipid Content of Plasma Globulin and Albumin Fractions.* Investigations of the phospholipids combined with the protein fractions of blood plasma were undertaken in view of the reported clot-aiding properties of the globulin[9] and the antithrombic potency of the albumin[10] fractions. Negligible amounts of phospholipid accompanied the globulins precipitated at pH 5.5 from dog, normal human, and hemophilic plasmas. Preliminary analyses of the globulin fraction precipitated with sodium sulfate, sodium sulfite,

† We appreciate the courtesy of J. H. Northrop for the supply of crystalline trypsin.

8 Lee, P., and Erickson, B. N., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 264.

9 Patek, A. J., and Taylor, F. H. L., *J. Clin. Inv.*, 1937, **16**, 113.

10 Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

and ammonium sulfate led to the prompt discovery that the accompanying phospholipid varied from none to half of the total plasma phospholipid depending upon the salting agent employed. The results demonstrate the necessity of separating the individual protein fractions with minimal chemical alteration, before information can be secured concerning the protein-phospholipid complexes of blood plasma.

*Summary.* The phospholipid partition (lecithin, cephalin and sphingomyelin) of hemophilic plasma revealed no lack of the clot-aiding phospholipid cephalin.

A tested technic showed only minute traces of "free" phospholipid in normal and hemophilic plasma. Measurable amounts of uncombined cephalin and other phospholipids were found, however, in lipemic plasmas. Attempts to liberate cephalin by tryptic digestion of plasma were unsuccessful. Added cephalin combined with the plasma proteins to a large extent, except in lipemias.

The lysis of platelets in the presence of calcium (yielding an active thromboplastic solution) gave lysates containing little or no phospholipid, free or combined.

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## Effect of Alpha-Tocopherol on Lesions of Skeletal Muscles in Rats on Vitamin A-Deficient Diets.

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Degenerative lesions of skeletal muscles have been described in rats on vitamin A-deficient diets.[1] We too have constantly observed such lesions in our experimental rats on a standard vitamin A-free diet.

In recent years muscular dystrophy in rats has been shown to be related to a deficiency in vitamin E. It was first produced in the suckling young of mother rats placed on a diet deficient in vitamin E by Evans and Burr[2] and the nature of the lesions in the muscles of the affected young were first described by Olcott[3] and recently by

1 Bessey, O. A., and Wolbach, S. B., "The Vitamins"—a Symposium, American Medical Association, 1939.

2 Evans, H. M., and Burr, G. O., *J. Biol. Chem.*, 1928, **76**, 273.

3 Olcott, H. S., *J. Nutrition*, 1938, **15**, 221.