oil, *per se,* on the progress of tuberculous infection.

Schoenheimer[9] has shown that the fatty acids of the diet are first incorporated into body fat before they are oxidized. Thus, the body fat is part of a dynamic system, the fatty acids being continually deposited and withdrawn.

Franke, *et al.*,[10] found that of all the substrates tested manometrically, certain of the fatty acids effected the greatest increase in respiration of *Mycobacterium tuberculosis* despite the fact that other of these acids were inhibitory under such conditions.

Due to the fact that *Mycobacterium tuberculosis* is located extra-cellularly in its host, this microorganism has access to the body fluids of the host. Since the body fat is in a dynamic state, such fluids will contain fatty acids corresponding to those of the diet. If the diet contains large amounts of fatty acids which serve to increase the respiration of *Mycobacterium tuberculosis,* enhanced proliferation of the organism would be expected to occur. However, if the diet contains large amounts of fatty acids which serve to inhibit the respiration of *Mycobacterium tuberculosis,* a retarded proliferation of the organism would be expected to result.

Thus, the type of fatty acids present in the diet may determine whether or not conditions are favorable for the development of tuberculous infection.

Summary. 1. The administration of a non-lipid, casein-supplemented ration to resistant Swiss albino mice retarded the progress of experimental tuberculosis. 2. Experimental tuberculosis was also retarded by the administration of the ration to which 20% of the total fatty acids of coconut oil had been added. 3. Olive oil, linseed oil, and oleic acid enhanced the progress of experimental tuberculosis when these compounds were added to the ration in a concentration of 20%.

[9] Schoenheimer, R., and Rittenberg, D., *Jour. Biol. Chem.*, 1936, **114**, 381.

[10] Franke, W., and Schillinger, A., *Biochem. Z.*, 1944, **316**, 313.

16408

A Lipid Anticoagulant from Brain Tissue. Physiochemical Characteristics and Action *in vitro* and *in vivo.**

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Dilution of a crude aqueous brain extract or of a suspension of its lipid (cephalin) fractions often improve their clot accelerating power. This apparent paradoxical behavior of strong thromboplastic solutions has been frequently observed.[1,2,3] Attempts to separate the clot inhibiting from the clot accelerating substances have only been moderately successful.[3,4] The experiments here reported indicate that it is possible to separate from brain tissue, by a suitable method of extraction, a heat labile inhibitor of blood coagulation, probably of a lipid nature and acting *in vitro* and *in vivo* as a powerful antithromboplastin.

Reagents. Thromboplastin. 300 mg of acetone dried human brain powder are extracted for ½ hour with 5 cc of 0.85% sodium chloride at 50°C. The supernatant fluid is centrifuged at 1000 R.P.M. for 2 minutes and tested against recalcified citrated plasma.

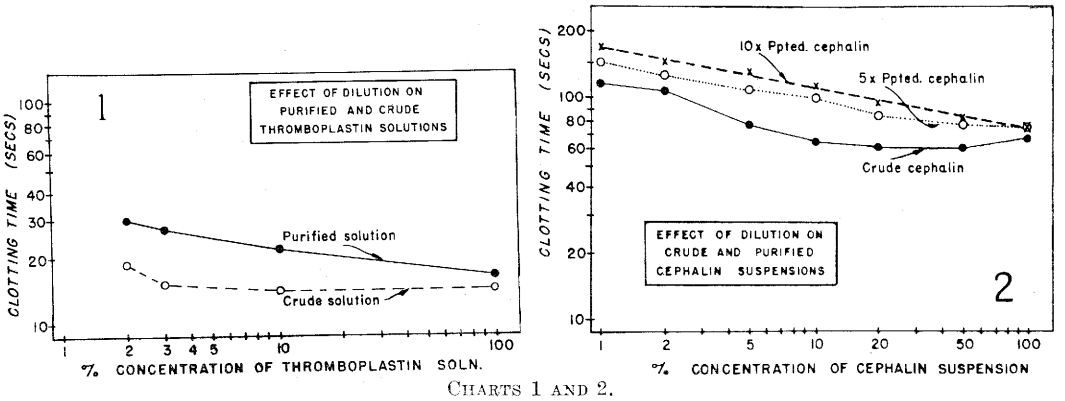
* Aided by grant from the U. S. Public Health Service.

[1] Aggeler, P. M., and Lucia, S. F., *Am. J. Med. Sci.*, 1940, **199**, 181.

[2] Kazal, L. A., and Arnow, L. E., *Arch. Biochem.*, 1944, **4**, 185.

[3] Chargaff, E., *J. Biol. Chem.*, 1937, **121**, 175.

[4] De Suto Nagy, G. J., *J. Biol. Chem.*, 1944, **156**, 433.



CHARTS 1 AND 2.

The clot accelerating action of intact and diluted thromboplastin and cephalin extracts. 0.1 cc of thromboplastin (or cephalin), 0.1 cc plasma, 0.1 cc 0.02 M $CaCl_2$.

Thromboplastin solutions were purified by repeated precipitations of the thromboplastic lipoprotein by dilution and acidification.[5] Cephalin suspensions were prepared by a method previously described; cephalin was purified by repeated precipitations of the lipid in its ether solution with cold absolute ethanol.[6] Unless otherwise stated, all tests were carried out in collodion coated tubes, 13 mm i.d. at 38°C. Citrated (0.38%) human plasma was collected with the precautions outlined elsewhere[7] and kept in paraffin tubes until tested.

1. *Activity of crude and purified aqueous thromboplastin solutions and cephalin suspensions after dilution.* As shown in Charts 1 and 2, crude preparations of thromboplastin and cephalin gain in clot accelerating potency on dilution, until a point is reached beyond which further dilution results in loss of clot promoting power. Purified preparations, however, though originally less potent than the crude ones, do not gain clot accelerating power upon dilution. It would seem, therefore, that during the course of purification a clot inhibitor is removed.

2. *Separation of the Anticoagulant Fraction from Brain Tissue.* The methods employed take advantage of the solubility characteristics of the clotting inhibitor and avoid any procedure which may involve raising the temperature beyond 50°C, since it was found early in the work that the inhibitor is heat labile.

First Method. 30 g of acetone dried human brain powder capable of passing through a 20-mesh sieve are extracted with 450 ml of absolute ether, in the cold, for 7 days with occasional shaking. The supernatant is filtered and concentrated *in vacuo* to a volume of 50-75 ml. To this sample is added sufficient cold absolute ethanol for maximum precipitation (approx. 125-150 ml). After standing in the refrigerator for 30 minutes it is centrifuged at 1500 R.P.M. for 5 minutes. The supernatant is decanted off and saved. The residue is dissolved in 20 ml of absolute ethyl ether. The precipitation with cold absolute ethanol is repeated, this time adding 110-125 ml of the alcohol. This is handled as before, again saving the supernatant and dissolving the residue in 20 ml of ether. A third precipitation is done, the combined supernatants are filtered and the filtrate distilled *in vacuo* in a flask immersed in a water bath maintained at 46-48°C. The residue remaining in the flask after the distillation is completed is removed with absolute ethyl ether. The ether is evaporated off, 50 ml of acetone added to the residue and the mixture is allowed to remain in contact at 5°C overnight. The acetone is decanted off and the acetone insoluble residue is dried *in vacuo*. A golden yellow powder results to which is

[5] Seegers, W. H., Brinkhous, K. M., Smith, H. P., and Warner, E. D., *J. Biol. Chem.*, 1938, **126**, 91.

[6] Tocantins, L. M., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 44.

[7] Tocantins, L. M., *Am. J. Physiol.*, 1943, **139**, 265.

TABLE I.
Relative Anticoagulating Potency of Lipid Inhibitor Fractions Obtained by Different Methods.

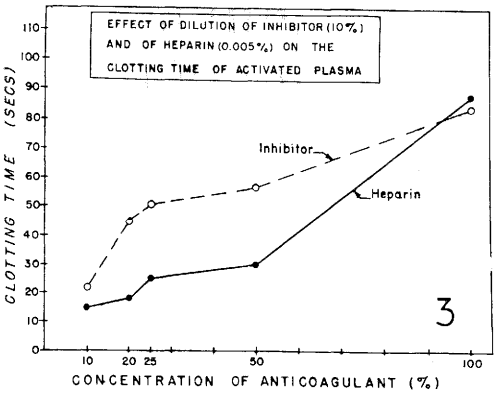
	Lipid inhibitor fractions				
	1st method	2nd method			Control
	Lot A	Lot B	Lot C		NaCl
Strength of solution, g %	50	10	10	50	0.85
Duration of extraction (days)	7	1	5-6		
Temp. of extraction, °C	5	20-22	5		
Heparin units/mg	0.25	0.5	0.75		
Clotting time:					
(a) Plasma + inhibitor sol. + $CaCl_2$	>24 hr	>24 hr	>24 hr	>24 hr	—
(b) Plasma + thrombopl. + inhib. sol. + $CaCl_2$	160 sec.	85 sec.	380 sec.	>24 hr	
(c) Plasma + $CaCl_2$ + 0.85% NaCl	—	—	—	—	500-610 sec.
(d) Plasma + thrombopl. + $CaCl_2$ + 0.85% NaCl	—	—	—	—	14 sec.

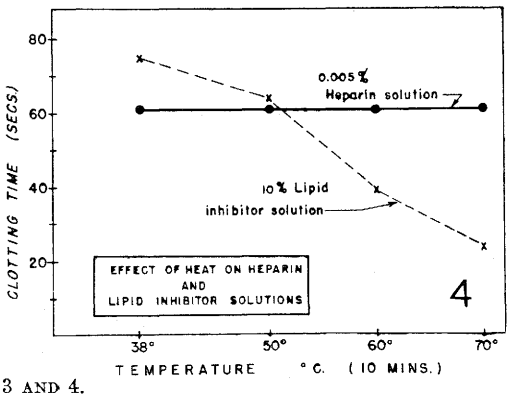
added 30 ml 0.85% NaCl. This is macerated with a mortar and pestle to make the solution as uniform as possible and then homogenized by putting through a colloidal mill 7-10 times. The pH of the solution is adjusted to 7.2-7.4 and it is then ready for use. The acetone soluble portion, on evaporation, yields a white powdery substance which has clot accelerating activity.

Second Method. 30 g of acetone dried brain powder are extracted for 5-6 days at 5°C with 600 ml of absolute methanol. The supernatant is filtered and the filtrate distilled *in vacuo* in a flask immersed in a water bath at 38-40°C. The residue remaining in the flask after the distillation is completed is removed with absolute ethyl ether. The ether solution is kept at 5°C overnight during which a white precipitate settles out. The supernatant is decanted off and the precipitate washed with cold ether. The combined ether extracts are evaporated off *in vacuo* leaving a creamy white, waxy powder. A 10 g% solution is made in 0.85% NaCl, put through a homogenizer 7-10 times and the pH adjusted to 7.2-7.4. The potency of the inhibitor seems to be related to the proportion of clot accelerating substances present and the latter seems to go in solution better at temperatures higher than 5°C.

The results of testing the activity of 3 lots obtained by the 2 methods above described are shown in Table I. The anticoagulating potency of the inhibitor has been compared with that of heparin (Connaught Laboratories, Toronto) 1 mg of which contains 100 units. Though the mechanism of action of the lipid inhibitor differs from that of heparin, comparison with it provides a general measure of its anticoagulant potency, in terms of that of a well known substance. Our most potent products have 0.75 heparin units per mg (Table I) or an activity 133 times less than that of crystalline heparin. Comparison of the 2 anticoagulants was done by noting their relative potency in delaying coagulation of recalcified citrated plasma activated by a strong thromboplastin solution. If 10 mg of the lipid inhibitor prolongs the clotting time of activated plasma from 14 to 80 seconds and this prolongation corresponds to that brought about by 5 units (or 0.05 mg) of heparin, then 1 mg of the lipid inhibitor may be considered to have ½ heparin unit. That this is only a rough method of comparison is shown in Chart 3. Dilution of the 2 anticoagulants shows that in the lower concentrations, the lipid inhibitor is a more effective antithromboplastin than the heparin.

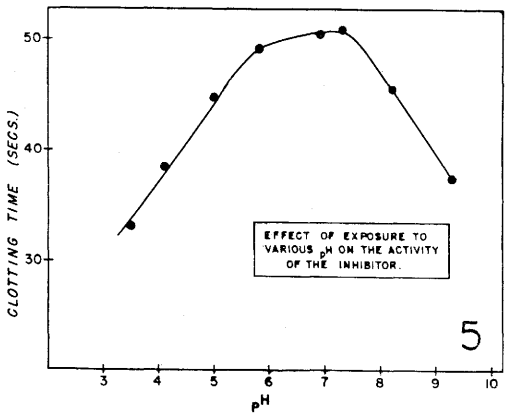
3. *Effect of Heat and pH Changes.* Unlike heparin, the lipid inhibitor is heat labile. Temperatures of 65-70°C for 10 minutes almost entirely destroy the activity of the inhibitor in 0.85% NaCl (Chart 4). Exposure





CHARTS 3 AND 4.

Comparison of the effect of dilution or heat on the action of heparin and the lipid inhibitor. 0.1 cc thromboplastin, 0.1 cc heparin (or inhibitor), 0.1 cc plasma, 0.1 cc 0.02 M $CaCl_2$.



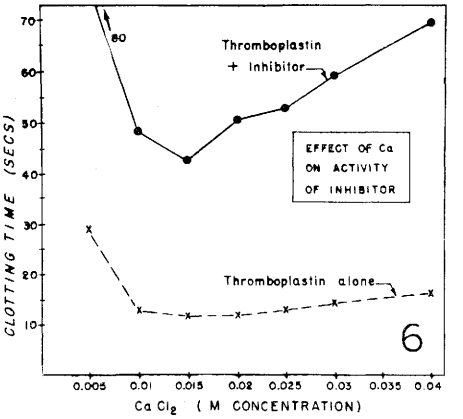


CHART 5.

The pH of a 10% solution of the inhibitor was kept at various levels for 180 min. by addition of dilute HCl or NaOH. It was then brought back to 7.2 and tested.

CHART 6.

0.1 cc thromboplastin, 0.1 cc 10% lipid inhibitor (or 0.85% NaCl), 0.1 cc plasma, 0.1 cc $CaCl_2$ (various M concentrations). The optimum range of Ca concentration is narrower for the clotting system containing the inhibitor, but in other respects does not differ from the system without the inhibitor.

to an excessively acid or alkaline medium likewise reduces the anticoagulating potency of the lipid inhibitor (Chart 5).

4. *Mode of Action.* The lipid extract seems to owe its clot delaying action to a reduction of the activating effect of thromboplastin on prothrombin (Table II). In contrast with heparin, it has no effect on thrombin even when a 20% solution of the lipid inhibitor is used (Table II). It does not seem to bind calcium (Chart 6) and does not alter the rate of conversion of fibrinogen to fibrin by thrombin. Since an antiprothrombic effect is difficult to distinguish from an antithromboplastic one, it is not possible to be certain in this regard. The fact that it does not block the activation of prothrombin by Russell viper venom may indicate that it does not have an antiprothrombin effect. The natural plasma antithromboplastin is likewise impotent against the clot accelerating action of Russell viper venom.[8]

5. *Physiochemical Characteristics.* The general physical and chemical characteristics of this inhibitor as prepared by Method 2 are as follows: It is a cream colored waxy ma-

8 Tocantins, L. M., *Am. J. Physiol.*, 1945, **143**, 67.

TABLE II.
Effect of Human Lipid Inhibitor on Human Plasma Activated by Human Thromboplastin or by Human Thrombin.

Lipid inhibitor (mg)	Clotting time (sec.)					
	Undilut. thrombopl.*	Dil. thrombopl.*	Undilut. thrombin†		Dil. thrombin†	
			0 inc.	10 min. inc.	0 inc.	10 min. inc.
0	14	21	20	694	39	1408
5	117	337	19	580	37	960
10	350	1348	19	520	36	744
20	658	>24 hr	19	450	36	528
50	>24 hr					

* 0.1 cc plasma, 0.1 cc inhibitor solution (or 0.85% NaCl), 0.1 cc thromboplastin, 0.1 cc 0.02 M $CaCl_2$.
† 0.1 cc heated (56°C for 5 min.) plasma, 0.1 cc inhibitor solution (or 0.85% NaCl), 0.1 cc thrombin solution (0 or 10 min. incubation), 0.1 cc fresh plasma. Thrombin prepared by the method described by Eagle.[13]

terial, soluble readily in ether, methyl alcohol, chloroform, carbon tetrachloride, benzene, pyridine, glacial acetic acid and warm 95% ethyl alcohol. It is insoluble in acetone and cold 95% ethyl alcohol. The lipid inhibitor described by Chargaff[3] is only slightly soluble in cold pyridine and ether and is recrystallizable from methyl alcohol. Our inhibitor gives a negative Molisch test, and negative reactions to various qualitative protein tests (biuret, xanthoproteic, Acree-Rosenheim). It is not affected by oxidation or drying at room temperature. In the less pure state (Method 1), its potency can be increased by exposure to ultra-violet light. However, this property diminishes as purification proceeds, possibly indicating the removal of a coagulant impurity susceptible to the rays. That the inhibitor is still in an impure state is indicated by its melting point, which ranges from 182-200°C. In a 10% solution in 0.85% NaCl it has a pH of 6.0-6.1. It appears to have optimum activity at a pH of 7.0-7.3.

6. *Species Preferential Action.* Thromboplastins from human, rabbit and mouse brain are most active in the plasma of the corresponding species (Table III). Moreover, human lipid inhibitor is most effective against

TABLE III.
Comparison of the *in Vitro* and *in Vivo* Action of Thromboplastins and Inhibitors Prepared from Various Species.

	Effect *in vitro**			Effect *in vivo*† Thromboplastin activity (units per cc)
	Human plasma	Rabbit plasma	Mouse plasma	
	Clotting time in seconds			
1. (a) Human thromboplast.	14	14	15	120
(b) Human inhibitor '' thromboplast.	164	93	18	120
2. (a) Rabbit thromboplast.	14	7	13	—
(b) Rabbit inhibitor '' thromboplast.	122	37	32	—
3. (a) Mouse thromboplast.	72	22	9	260
(b) Mouse inhibitor '' thromboplast.	1850	104	38	200

* (a) 0.1 cc plasma, 0.1 cc thromboplastin, 0.1 cc 0.85% NaCl, 0.1 cc 0.02 M $CaCl_2$. (b) 0.1 cc plasma, 0.1 cc thromboplastin, 0.1 cc inhibitor, 0.1 cc 0.02 M $CaCl_2$.
† (a) One unit is equivalent to the minimum lethal dose, or the minimum amount of thromboplastin solution which when injected intravenously will kill within 30 min., at least one and produce convulsions in at least one other, out of five 20-25 mice. Total volume of injected material 0.25 cc, injected within 3 sec.
(b) Inhibitor suspension 0.12 cc, injected intravenously 1-2 min. before injection of 0.12 cc of the thromboplastin solution.

human thromboplastin when tested in its own plasma, less effective when tested in rabbit plasma and almost ineffective when mouse plasma is used (Exp. 1, Table III). Rabbit inhibitor likewise seems to exert its greatest antithromboplastic effect in human plasma and least in mouse plasma (Exp. 2, Table III). Mouse inhibitor, however, exerts a greater effect against its own thromboplastin in human and rabbit plasma than in its own plasma (Exp. 3, Table III). This may indicate that a cofactor in plasma is necessary for the antithromboplastin action of the inhibitor. Human plasma would seem to have a greater amount of this cofactor than rabbit plasma, and rabbit plasma in turn more than mouse plasma.

Like Schneider[9] we have found that when an undiluted solution of human thromboplastin is injected intravenously into mice, the animals usually die from intravascular clotting within about 3 minutes after the injection. Most human brain thromboplastin solutions contain from 80 to 160 *in vivo* thromboplastin units per cc (Table III). When equal amounts of human lipid inhibitor and human thromboplastin are injected into mice intravenously, 1-2 minutes apart, the minimal lethal dose of the thromboplastin is unchanged. Mouse brain thromboplastins have contained from 160 to 260 *in vivo* units per cc of solution. If before an intravenous injection of the thromboplastin, an adequate amount of the mouse inhibitor is injected, the potency of the thromboplastin is reduced (Table III). Some mice are completely protected against weak mouse thromboplastin solutions by a preliminary injection (1-2 minutes before) of the lipid inhibitor. It seems, therefore, that we find *in vivo* the same species preferential activity of the inhibitor as *in vitro*. The human inhibitor cannot protect the mouse against an injection of human thromboplastin. The mouse inhibitor, however, can protect the mouse against an injection of its own thromboplastin.

7. *Anticoagulating Effect in vivo.* Lipid inhibitor solutions prepared from rabbit brain were injected intravenously into rabbits (600 mg per kg body weight). There were no apparent constitutional reactions, except for difficulty in checking bleeding from wounds made for collection of blood samples. In Chart 7 are 2 examples of a comparison of the results of such an injection with that of heparin (300 units, or 3 mg per kg body weight). One week was allowed to elapse between injections of the 2 anticoagulants and

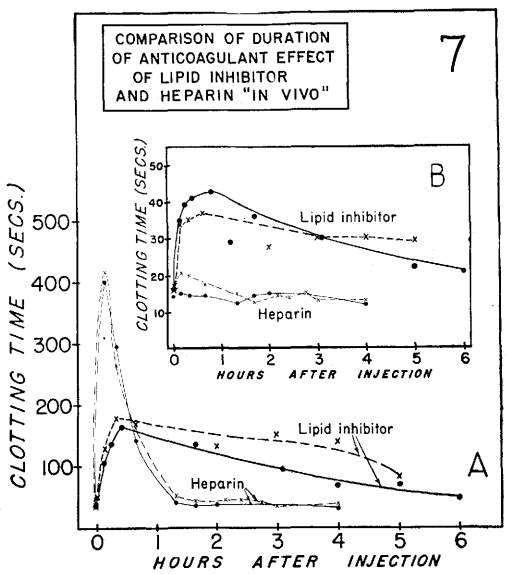


CHART 7.

Clotting times of two rabbits after intravenous injection of the lipid inhibitor or of heparin. A—Clotting times of 20 cu mm of blood (ear vein) to which 20 cu mm of a diluted solution of rabbit brain thromboplastin was added. B—Clotting times of 20 cu mm of blood to which 20 cu mm of an undiluted solution of the same thromboplastin was added. Readings on glass surfaces at 20°C.

the same animals were used for both experiments. The doses were selected on the basis of the relative potency of the 2 anticoagulants *in vitro* against activated plasma (1 mg lipid inhibitor = ½ unit heparin). The curves demonstrate that though heparin may exert a greater anticoagulant action when dilute thromboplastin is used as test solution, its effect is dissipated within 1½ hours, while some evidence of activity of the lipid inhibitor is still detectable 5-6 hours after injection. Moreover, the lipid inhibitor seems to exert a greater action than heparin against the stronger thromboplastin solution. In the

[9] Schneider, C. L., *Am. J. Physiol.*, 1947, **149**, 123.

TABLE IV.
Comparison of Source, Potency, and Other Characteristics of Four Inhibitors of Blood Coagulation.

Anticoagulant:	Heparin (Toronto)	Chargaff 1937[3]	De Suto Nagy 1944[4,10]	Tocantins, Carroll and McBride 1948[14]
Source:	Beef lung	Sheep and pig brain	Pig spleen	Human brain
Chemical nature:	Sulfuric acid ester	Lipid	Protein lipid complex	Lipid
Behavior to heat:	Not destroyed by boiling	Not destroyed in boiling alcohol	Not destroyed in boiling alcohol	Destroyed after 10 min. 65-70°C
Mode of action:				
Antithromboplastin	Yes	—	Yes	Yes
Antithrombin	Yes	—	Yes	No
Antifibrinogen	No	—	No	No
Calcium binding	No	—	No	No
Concentration of solution:	0.005%			10%
Clotting time:				
Plasma + $CaCl_2$	250-350 sec.	120 sec.	348 sec.	250-350 sec.*
Plas. + anticoag. + $CaCl_2$	>24 hr	4920 "	>1800 "	>24 hr*
Plas. + thrombopl. + $CaCl_2$	12 sec.		9 "	12 sec.*
Plas. + thrombopl. + anticoag. + $CaCl_2$	81 "		9 "	280 " *
Source of plasma:	Man	Man	Dog	Man

* Figures quoted were obtained in tests carried out in glass tubes to make it possible to compare our findings with those of other workers.

quantities used, heparin does not seem to be *in vivo* as strongly antithromboplastic as the lipid inhibitor (Chart 7).

Comment. The lipid inhibitor of blood coagulation here reported has some similarities with that described by Chargaff,[3] but unlike it, our inhibitor is extracted in the cold, is heat labile and easily soluble in ether. It does not seem to be the same substance discussed by De Suto Nagy,[4,10] for it has widely different chemical properties and mode of action. Our inhibitor has greater potency than either of the other 2 substances (Table IV).

The fluidity of circulating blood seems to be maintained by a balance between anticoagulant and coagulant factors in the blood and surrounding tissues.[11] Whether or not the inhibitor here reported or a similar one play a role in aiding to maintain this fluidity remains to be clarified by further work. Thromboplastic substances liberated from damaged tissues and blood cells disrupt this equilibrium and initiate the changes (first phase of coagulation) which lead to clotting. The natural antithromboplastin of the plasma slows the development of clotting by reducing the amount of thromboplastin available for the activation of prothrombin. An antithromboplastin substance has been extracted from the plasma with methanol; when the methanol extract is added to the plasma, it enhances its antithromboplastic activity.[12] The lipid inhibitor here described is also soluble in methanol and has, furthermore, a range of thermolability similar to that of the natural antithromboplastin of the plasma.[7]

The necessity of a plasma cofactor for the development of the antithromboplastic effect of the lipid extract may explain why inhibitors and accelerators of coagulation may exist side by side in the tissues without neutralizing each other's action. The lipid inhibitor becomes an effective antagonist to its own thromboplastin, only in the presence of a plasma cofactor. Further observations on the nature of this cofactor and the species preferential activity of the lipid inhibitor are

[10] De Suto Nagy, G. J., *Am. J. Physiol.*, 1944, **141**, 338.

[11] Tocantins, L. M., *Blood*, 1947, **1**, 156.

[12] Carroll, R. T., and Tocantins, L. M., to be published.

being reported in greater detail elsewhere. The data already presented, however, serve to stress the necessity of avoiding the mixing of clotting reagents derived from different species. This is particularly applicable to thromboplastins and antithromboplastins, a precaution that has been stressed by us[7] and others.[13]

No direct correlation seems to exist between the effectiveness of heparin and the lipid inhibitor as tested *in vivo* and *in vitro*. While heparin is the stronger anticoagulant, the fact that it is rapidly excreted or inactivated *in vivo* has seriously limited its clinical usefulness. The longer duration of the effect of an intravenous injection of the lipid anticoagulant when compared with that of heparin seems to make the lipid more suitable in the anticoagulant therapy of thromboembolic disease.[14]

Summary. A heat labile lipid inhibitor of blood coagulation has been extracted from brain tissue, differing in potency and other respects from similar known anticoagulants. It has a pronounced antithromboplastic activity especially against homologous brain extracts in homologous plasma. Though the anticoagulant power of this lipid antithromboplastin *in vitro* is still less than that of heparin, it has a more lasting effect when injected intravenously than a solution of heparin of equivalent potency.

[13] Fantl, P., and Nance, M. A., *Med. J. Australia*, 1946, **2**, 125.

[14] Tocantins, L. M., Carroll, R. T., and McBride, T. G., *Fed. Proc.*, 1948, **7**, 125.

16409

Blood Volume and Sympathectomy in Hypertension.

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It has been observed that in hypertensive patients following sympathectomy there frequently is a return of blood pressure to the preoperative level after a brief period of relative hypotension. Since blood volume is an important factor in maintenance of the blood pressure level, it seemed advisable to investigate the possible changes in blood volume consequent to the postulated enlargement of the vascular bed produced by sympathectomy and to correlate these changes with those in the blood presure. Our results indicate that there are no consistent changes in total blood volume or in plasma volume during periods as long as 18 months after operation. Immediate postoperative reduction in red cell mass with concomitant increase in plasma volume occurred frequently and persisted in many cases. The postoperative prognosis in patients with low red cell mass preoperatively seemed to be poor.

Adequate postoperative determinations were available for 20 patients, 11 of whom were women and 9 men, ranging in age from 22 to 50 years. Follow-up observations ranged from 3 to 18 months. Thoracolumbar sympathectomy was done in all cases except one in which the transthoracic approach was used. Plasma volumes were measured photocolorimetrically with the T-1824 dye according to the procedures outlined by Gregersen.[1] Hematocrit readings were obtained by the Wintrobe method and these values were employed to calculate whole blood and red cell volumes. Standard values of 45 cc/kg were used for the plasma volumes of men, 40 cc/kg for women and 40 and 35 cc/kg for red cell volumes in men and women, respectively.

[1] Gregersen, M. I., *J. Lab. and Clin. Med.*, 1944, **29**, 1266.