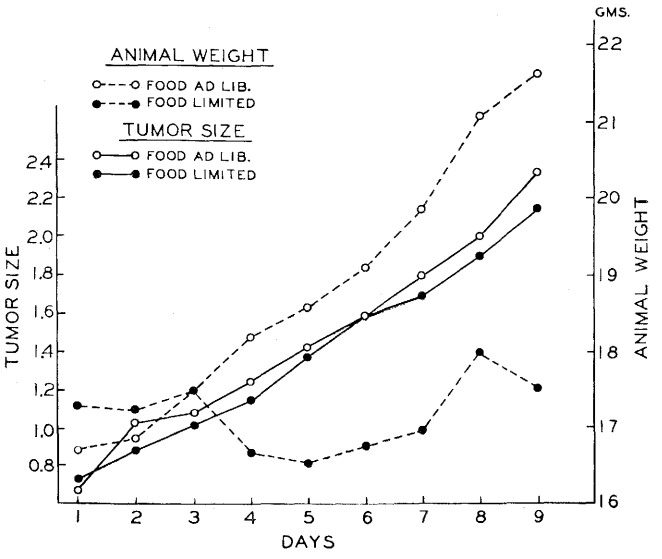

FIG. 3.
Food limitation was begun on the second day.

glutamic acid) daily for 5 days. The growth curve of the tumor in these two groups was not significantly different from controls treated with phosphate buffer alone, or from the growth curves when "synthetic folic acid" was used.

The weights of the animals receiving both bis (β-chloroethyl) sulfide and folic acid were maintained better than those receiving bis (β-chloroethyl) sulfide alone. To exclude the possibility of weight loss associated with drug administration as the cause for the differences in tumor regression a paired feeding experiment was carried out, the results of which are shown in Fig. 3. Here it is shown that limitation of food to 60% of that eaten by the control mice has no measurable effect upon the tumor growth. The weight loss produced by the food limitation is comparable to that which is frequently encountered after bis (β-chloroethyl) sulfide administration. Additional studies are necessary to elucidate the mechanism of folic acid antagonism to bis (β-chloroethyl) sulfide.

Summary. "Synthetic folic acid", "Folvite," or "Teropterin" in the dosage employed do not produce regression in transplanted $6C_3HED$ tumors in C_3H mice. Administration of synthetic folic acid partially inhibits the effect of bis (β-chloroethyl) sulfide on lymphosarcoma $6C_3HED$.

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Activation of Staphylocoagulase.*

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Smith and Hale[1] have shown that the inability of staphylocoagulase to clot citrated plasmas is, in most cases, due to absence of an "activator substance" for the staphylo-

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coagulase. Gratia[2,3] originally showed that staphylocoagulase clotting is independent of prothrombin activation, and Rigdon's[4] recent evidence, using heparin, confirms the inability of antithrombic agents to inhibit staphylocoagulase clotting. We have repeatedly confirmed these facts and present the following data as a preliminary report of progress in identifying the "activator" in various plasma, serum, and other materials.

Materials and methods. 1. STAPH.: Staphylocoagulase was prepared by precipitating 48-hr. *Staphylococcus aureus* (stock strains) broth-culture filtrate (Berkefeld *N*), or centrifugate, with 3 volumes of 95% ethyl alcohol, at 0°C. The method is essentially that by which Tillett and Garner[5] prepared "fibrinolysin" (*streptokinase*[6]). The ability to obtain an active staphylocoagulase from simple broth culture filtrates confirms Smith and Hale[1] and opposes Lominski,[7] whose results were negative unless plasma was added to the culture medium. A typical yield from 850 cc broth culture filtrate approximates 1.5 g of grayish-brown powder, completely soluble in 1% solution in our citrated borate buffer. The powder, stored in refrigerator (5°C) for 8 months, showed no loss of activity and its solutions were stable for as long as 10 days at room temperature. Reagents previously described:[6] 2. buff. (cit.): borate buffer (plus 0.4% sod. citrate; pH: 7.55), 3. B.F.: (Armour's) bovine plasma fraction-I (50% fibrinogen), 4. H.F.: (Harvard) human plasma fraction-I (35% fibrinogen),‡ 5. PRO.: (Seegers') purified bovine prothrombin.

Additional reagents: 6. H.Pl.: human plasma, 'Lyovac' dried (Sharp and Dohme), 7. B.f.: bovine fibrinogen (Seegers[8]), 8. Ac.G.: "accelerator globulin" from bovine plasma, courtesy Dr. W. H. Seegers[9] (Wayne Univ.), 9. Factor V: (after Owren[10]) prepared from dog plasma, 10. H.Pl.G. and H.S.G.: human 'globulin,' fractionated by 50% sat. $(NH_4)_2SO_4$ from (a) H.Pl. (*v.* 6) and (b) serum from same after clotting with $CaCl_2$, 11. H.Pl.A. and H.S.A.: human plasma (a) and serum (b) 'albumin,' fractionated from supernatants of above at 100% sat. $(NH_4)_2SO_4$, 12. H.S.A. cryst.: crystalline human serum albumin (Harvard Labs.),‡ 13. tpln.: commercial thromboplastins—I. Difco, II. Maltine, prepared according to directions.

Results. Table I describes staphylocoagulase tests with human plasma and the globulin and albumin fractions from plasma and serum. Bovine fraction-I (fibrinogen), plasma, and buffer (without STAPH.) do not clot (2) and B.F. has no activator substance (3), which must be supplied by the addition of plasma (1). The globulins from plasma (5) or serum (9) contain a weak thrombin, but the definite clotting improvement in the presence of staphylocoagulase (4,8) indicates some activator substance also. Crude albumins from plasma (6) or serum (10) are the best single source of activator substance, and are not coagulant in the absence of staphylocoagulase (7,11). Further investigation of these albumin fractions is currently being undertaken to yield more information on the staphylocoagulase mechanism.

Table II lists other substances tested by the same means as in Table I, except for

1 Smith, W., and Hale, J. H., *Brit. J. Exp. Path.*, 1944, **25**, 101.

2 Gratia, A., *C. R. Soc. Biol.*, 1919, **82**, 1245, 1247, 1393.

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5 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485; 1934, **60**, 239.

6 Ferguson, J. H., Travis, B. L., and Gerheim, E. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 285, 302.

7 Lominski, I., *Nature*, 1944, **154**, 640.

‡ The human plasma fractions used in this work were prepared from blood collected by the American Red Cross under a contract between the Office of Scientific Research and Development and Harvard University, and supplied through the courtesy of Drs. Cohn, Edsall, Minot, and colleagues.

8 Ware, A. G., Guest, M. M., and Seegers, W. H., *Arch. Biochem.*, 1947, **13**, 231.

9 Ware, A. G., Guest, M. M., and Seegers, W. H., *J. Biol. Chem.*, 1947, **169**, 231.

10 Owren, P. A., *The Coagulation of Blood: Investigations on a New Clotting Factor* (Oslo), 1947, p. 327.

TABLE I.
Staphylocoagulase Tests with Plasma and Serum Fractions.

No.	B.F. (1%) (0.5 cc)	Buff. (cit.) (0.25 cc)	Staph.† (0.25 cc)	Plasma fraction (0.5 cc)	Clotting-times (min., at 39°C)* 1+	2+	3+	4+
1	+	—	+	H.Pl.	21′	78′	—	107′
2	+	+	—	H.Pl.	No clotting			
3	+	+	+	—	,,	,,		
4	+	—	+	H.Pl.G.	13′	—	78′	163′
5	+	+	—	H.Pl.G.	40′	(clot never complete)		
6	+	—	+	H.Pl.A.	21′	—	33′	46′
7	+	+	—	H.Pl.A.	No clotting			
8	+	—	+	H.S.G.	—	—	21′	42′
9	+	+	—	H.S.G.	—	—	21′	92′
10	+	—	+	H.S.A.	—	—	—	17′
11	+	+	—	H.S.A.	No clotting			

† Staph.: Lot No. 5-6.
* Degree of clotting expressed as follows:
1+: first appearance of weak clot;
2+: weak clot with considerable liquid;
3+: good clot with a little liquid but too soft to invert tube;
4+: solid clot, tube invertible.

TABLE II.
Tests for "Activator Substance" of Staphylocoagulase.

No.	Material tested (0.5 cc)	B.F. (1%) (0.5 cc)	Staph.† (0.25 cc)	Clotting-times (39°C) 1+	4+
1	H.S.A., cryst. (1%)	+	+	No clotting (48 hr)	
2	PRO. (1%)	+	+	,,	,,
3	Factor V (1%)	+	+	,,	,,
4	Ac.G. (1%)	+	+	,,	,,
5	H.F. (1%)*	—	+	20′	40′
6	B.f. (1%)*	—	+	40′	overnight
7	tpln——I (5%)	+	+	—	240′
8	tpln.—II (2.5%)	+	+	—	60′

† Staph.: Lot No. 5-4.
* Double volumes (1.0 cc) of H.F. or B.f. used, and B.F. omitted.

substituting the respective material for the plasma fraction. The negative results with (1) *crystalline* human serum albumin, (2) purified prothrombin, and the recently identified globulin accelerators of prothrombin conversion (thrombic clotting), *viz.* (3) Owren's factor V and (4) Seegers' Ac.G., are noteworthy. The impure fibrinogens (5,6), which contain some activator substance, should be compared, in this respect, with B.F. (Table I, 3). With enough citrate in our test system to prevent possible thrombin formation (only negligible traces of prothrombin occur in B.F.,[6] however), the evidence for accelerator substance in the thromboplastins (7,8) is interesting.

Summary. The preparation and some properties of a stable staphylocoagulase are described, together with data to indicate that the "activator substance" which it needs in order to coagulate fibrinogen can be provided by certain plasma protein fractions, particularly being associated with the albumins.