thus obtained does not differ significantly from 100%.

Summary. Natural progesterone isolated from pigs' ovaries in 1936 shows no loss of activity after storage for 10 years in glass-stoppered vials at room temperature. Essentially similar methods of bioassay at widely separated intervals of time give dosage response curves that show no statistically significant differences.

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Relation of Complement to Blood Coagulation.

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Evidence has been presented both for and against the participation of complement in the coagulation of the blood. Wadsworth, Maltaner and Maltaner[1] favored the affirmative view because many substances were found to have both anticoagulant and anticomplementary actions. Maltaner[2] reported a close correlation between thromboplastic and complement-fixing power of tissue extracts and sera. On the other hand, Wilander[3] and Ecker and Pillemer[4] found great quantitative differences between the relative anticoagulant and anticomplementary properties of a number of substances. Quick[5] showed that complement as a whole is not the same as prothrombin. However, much recent work points to the existence of an additional component of the coagulation system, concerned with the conversion of prothrombin to thrombin. We[6] have already quoted much of this work but should mention in addition the contributions of Ware, Guest, and Seegers[7,8] and the extensive and convincing investigations of Owren.[9] A study of the role that complement plays in the conversion of prothrombin to thrombin appears timely.

Methods. Human plasma was freed of complement activity in 3 ways: by aging and by treatment with zymin and with ammonia. After aging for 2 to 3 months at icebox temperature plasma had no complement activity. Zymin powder was prepared from yeast essentially as described by Ecker, Jones, and Kuehn.[10] Two hundred and fifty milligrams of zymin were mixed with 1 cc of oxalated plasma and 1 cc of imidazole buffer at pH 7.2. Ordinarily serum is incubated with zymin for one hour at 37°C in order to inactivate complement, and smaller quantities than used by us suffice. We found that if the plasma was centrifuged immediately after thorough mixing with zymin, most but not all of the complement activity had disappeared by the time the supernatant was tested, about 20 minutes after the addition of zymin. The remaining complement activity disappeared gradually over a period of one to 2 hours in the absence of zymin. This was the procedure followed in preparing the zymin plasma used in the experiments described subsequently. This

1 Wadsworth, Augustus, Maltaner, Frank, and Maltaner, Elizabeth, *J. Immunol.*, 1937, **33**, 297.

2 Maltaner, Frank, PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 302.

3 Wilander, Olof, *Skandinav. Arch. f. Physiol.*, 1938, **81** (Suppl. 15), 89 pp.

4 Ecker, E. E., and Pillemer, L., *J. Immunol.*, 1941, **40**, 73.

5 Quick, A. J., *J. Immunol.*, 1935, **29**, 87.

6 Mann, F. D., Hurn, Margaret, and Magath, T. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 33.

7 Ware, A. G., Guest, M. M., and Seegers, W. H., *Science*, 1947, **106**, 41.

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

9 Owren, P. A., *The Coagulation of Blood; Investigations on a New Clotting Factor*, Oslo, J. Chr. Gundersen, Boktrykkeri, 1947, 327 pp.

10 Ecker, E. E., Jones, C. B., and Kuehn, A. O., *J. Immunol.*, 1941, **40**, 81.

TABLE I.
One-stage Assays: 0.1 cc of Standard Rabbit Brain Thromboplastin and 0.1 cc of 0.025 M Calcium Chloride Added to Tubes Containing 0.1 cc of Each of the Following Pairs of Components (Total Volume 0.4 cc).

Components present	Complement	Clotting time, sec.
Untreated plasma and saline	Present	19
Zymin plasma and saline	Absent	202
" " " serum	Present	20
NH_3 plasma and saline	Absent	190
" " " serum	Present	22
Zymin plasma and NH_3 plasma	"	32
Aged plasma and saline	Absent	186
" " " serum	Present	21
" " " zymin plasma	"	28
" " " NH_3 plasma	Absent	193

zymin-treated plasma, when mixed with four volumes of fresh oxalated plasma and incubated at 37°C, removed in several hours most of the complement activity of the added plasma. These observations, which suggest that the action of zymin is not one of simple adsorption, are being studied further. Ammonia-treated plasma was prepared by incubating the mixture of 1 cc oxalated plasma, 1 cc imidazole buffer and 0.2 cc of 1% ammonium hydroxide for one hour at 37°C. This mixture had a pH of 8.2 and approximately the minimal concentration of ammonia reported as necessary to inactivate the fourth component of complement in serum.[11] After the treatment with ammonia the plasma was devoid of complement activity. To test for complement 0.2 cc of treated plasma was added to 1 cc of a 1% suspension of sheep cells sensitized with 2 units of amboceptor and the mixture was incubated for one hour at 37°C. Serum was also inactivated by zymin and by ammonia by the same procedures used for plasma. Thrombin-forming activity was tested by a one-stage procedure using a system of 0.4 cc total volume[6] and by a 2-stage procedure,[12] both exactly as previously described except that the plasma was not defibrinated in the 2-stage method.

Results. In all 3 types of complement-inactive plasma, marked loss of thrombin-forming ability was observed for both one-stage and 2-stage methods. The rapid, marked increase in the one-stage prothrombin time on aging is, of course, well known; after prolonged aging, as in these experiments, the yield of thrombin with the 2-stage procedure is also very low. Striking decrease of the one-stage prothrombin time and increase of the 2-stage thrombin yield were obtained with all 3 types of treated plasma by use of 0.1 cc of serum in the 0.4 cc one-stage system and by addition of 0.1 cc of serum to the 2 cc of incubation mixture in the 2-stage procedure. The serum used (4 to 6 hours subsequent to spontaneous coagulation) did not clot fibrinogen and contained no fibrinogen and only very small amounts of prothrombin as shown by the 2-stage assay. Serum inactivated with zymin or ammonia did not restore thrombin-forming activity to similarly inactivated plasma. Mixtures of zymin plasma and aged plasma formed thrombin readily and had appreciable, although not normal, complement activity. Mixtures of aged plasma and ammonia plasma showed no restoration of thrombin formation and little or no complement activity. The addition of serum inactivated by heating at 56°C for 30 minutes partially restored complement activity to ammonia-treated plasma but did not restore thrombin-forming ability. An illustrative protocol (Tables I, II, and III, all data obtained with the same reagents on the same day) shows most of the effects observed. All of the foregoing experiments were easily reproducible.

Comment. The observation that plasma, by 3 different methods, may be almost completely deprived of its ability to form throm-

[11] Pillemer, L., Seifter, J., and Ecker, E. E., *J. Immunol.*, 1941, **40**, 89.

[12] Hurn, Margaret, and Mann, F. D., *Am. J. Clin. Path.* (Tech. Sect.), 1947, **17**, 741.

TABLE II.
Two-Stage Assays: Dilution of Various Plasmas Is That in the 2 cc of Incubation Mixture Containing Calcium and Thromboplastin. Units of Prothrombin Are Calculated in Terms of Original Plasma or Total Plasma When Aged Plasma Is Included. Amount of Serum Is That Added to the 2 cc Incubation Mixture. The Very Low Yields of Thrombin Give Clotting Times Too High for Accurate Determination.

Plasmas and dilutions		Serum, cc	Yield of thrombin, units per cc of plasma
Untreated	1:100	0	490
None		0.1	< 5
Zymin	1:40	0	Slight trace
"	1:40	0.1	280
NH_3	1:40	0	<20
"	1:40	0.1	290
Zymin	1:80 + NH_3 1:80	0	320
Aged	1:40	0	<20
"	1:80	0.1	300
"	1:80 + Zymin 1:80	0	300
"	1:80 + NH_3 1:80	0	<20

N.B. Units of prothrombin are determined in terms of yield of units of thrombin.

TABLE III.
Tests for Complement Activity: 1 cc Sensitized Sheep Cells Added to Each of the Following.

Components present	Hemolysis
.1 cc serum + 0.1 cc saline	Complete
.1 " untreated plasma + 0.1 cc saline	"
.2 cc zymin plasma	None
.2 " NH_3 "	"
.2 " aged "	"
.1 " zymin " + 0.1 cc NH_3 plasma	Partial
.1 cc zymin plasma + 0.1 cc aged plasma	"

bin while leaving abundant prothrombin which is highly reactive in the presence of the proper conversion factors, makes it somewhat doubtful whether any method of assay yet devised measures a single substance, prothrombin. The foregoing experiments point to a complex rather than a simple prothrombin conversion factor.

The inactivations of the third and fourth components of complement by zymin and by ammonia respectively are regarded as fairly specific, being in fact the bases for definition of these components. To regard the blood coagulation system and complement as closely related appears to be the point of view most likely to direct efforts toward increasing knowledge of both.

Summary. Inactivation of the complement of plasma by aging or by treatment with zymin or with ammonia blocks conversion of prothrombin to thrombin while leaving ample reactive prothrombin.

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Cultivation of Toxoplasma in Embryonated Egg. An Antigen Derived from Chorioallantoic Membrane.

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Neutralization[1] and complement-fixation[2] tests have been used for establishing the diagnosis of toxoplasmosis in man; however, neither method is entirely satisfactory. The

1 Sabin, A. B., and Ruchman, I., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 1.

2 Warren, J., and Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 11.