TABLE I.
Comparative Gains on Chicks on Diets Containing the Equivalent of 20% Crude Protein Exclusively from Sunflower Seed Meal, Sardine Meal, or Casein Plus Certain Amino Acids.

Exp. No.	Amino acid source	Chicks per group, No.	Duration of exp., days	Avg total gain, g	Rate of gain per day, %	Gain per g feed consumed, g
1	Sunflower seed meal, com'l.	5	11	62	5.3	
	Sardine meal*			67	5.7	
2	Same	6	12	121	5.7	0.36
				112	5.4	0.34
3	Sunflower seed meal, com'l.	10	21	147	4.8	0.38
	Casein, arginine, cystine, glycine			147	4.8	0.39
4	Sunflower seed meal†	10	9	76	6.5	0.42
	Sunflower seed meal,† lysine			80	6.5	0.43
	Casein, arginine, cystine, glycine			57	5.5	0.43

*This product was deboned by floating on carbon tetrachloride; it contained 85.0% protein (N × 6.25).
†Sample prepared in the laboratory from sunflower seed.

The amino acids which are required by the chick for optimal growth are greater in number and equal to or greater in quantity than those required by the rat.[5] The disagreement between the rat biological data and the amino acid and chick biological data may be due to the use of poor quality samples in the rat tests.

Summary. The protein of sunflower seed is a complete single source of amino acids for the growth of the young chick, when fed to provide 20% protein in the diet.

15195

"Thermostable" Thromboplastin from Human Placenta and Chicken Brain.*

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In the course of a study of the hormones of placenta we observed that extracts of this organ exhibit a strong blood-coagulating action.[1] The active substance resembled thromboplastin, the factor which by converting prothrombin into thrombin activates the reaction fibrinogen → fibrin and thus promotes the coagulation of blood. It could be shown that this active agent is relatively thermostable.

Heat stable thromboplastin from placenta is of special therapeutic interest, since its use makes it possible to avoid the side-effects which attend the local or parenteral administration of thromboplastin from heterogenic source.[2,3] Since thromboplastin of birds is less active on mammal blood than is mammal thromboplastin and vice versa, it seems probable that the two are different. A comparison between these two thromboplastins was therefore carried out.

The properties of thromboplastin have been studied in the last few years by various laboratories. A very extensive research has been

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1 Finkelstein, M., *Nature*, 1945, **155**, 202.

2 Eley, R. C., Green, A. A., and McKhann, C. F., *J. Pediat.*, 1936, **8**, 135.

3 Edsall, Y. T., Ferry, R. M., and Armstrong, S. H., *J. Clin. Invest.*, 1944, **23**, 557.

undertaken by Chargaff *et al.*[4-8] These authors isolated substances of a thromboplastic nature from animal tissues by a variety of methods. Using water as extracting medium and purification of the extract by differential centrifugation procedures they obtained thromboplastic preparations described as "high molecular lipoprotein." By means of organic solvents thromboplastic preparations belonging to the phosphatide group were separated. Both preparations proved relatively thermostable.[5,6]

Experimental. Preparation of Thromboplastin from Placenta. Placenta was perfused with tap-water and then minced with sand. 20 g of the mince was extracted with 20 cc cold distilled water. The suspension was centrifuged. The supernatant fluid was sucked through a Seitz filter, and the filter pad was then washed with distilled water until the wash-water became colorless. The filter pad was broken up under cold distilled water and the suspension was filtered through filter paper. The resulting almost colorless, opalescent filtrate contained the thromboplastin used in the present study. It contained 1.4 mg dry matter per cc and exhibited a protein reaction with sulfo-salicylic acid.

Preparation of Thromboplastin from Chicken Brain. Twenty g of chicken brain was freed from grossly visible blood vessels and minced in a mortar with sand in 20 cc of water. The mash was pressed through gauze. After centrifugation the supernatant was diluted with equal parts of distilled cold water and was centrifuged again. The upper layer was removed with a pipette and filtered through a Seitz filter. Subsequent steps were as described for the preparation of thromboplastin from placenta. The resulting solution contained 1 mg dry matter per cc.

The thromboplastic activity of the preparation from placenta was tested on oxalated human plasma, and the preparation from chicken brain was tested on native† chicken or rooster plasma. (The thromboplastin from chicken brain shows very little activity when tested on human oxalated plasma, and high activity when tested on chicken plasma; thromboplastin from human sources exhibits the reverse behavior.)

The "coagulation time" of oxalated human plasma was tested by the addition of 0.1 cc thromboplastin to 0.1 cc plasma. After 5 minutes of incubation at 37°C 0.1 cc 0.02 M calcium chloride was added and the tube was shaken vigorously. The time of clot formation was recorded with a stop watch.

For the determination of the "coagulation time" of chicken plasma 0.1 cc of chicken plasma was transferred to a small clean tube kept in a water bath at 37°C. After 5 minutes of incubation 0.05 cc of thromboplastin was added with a micropipette and the tube was shaken vigorously. The time from the addition of the thromboplastin until clot formation was noted with a stop watch. Every experiment was performed at least 3 times. Only plasma specimens whose coagulation times without thromboplastin were not less than 2 hours were used.

Results. As we have reported in a preliminary paper[1] the purified aqueous placenta extract with strong thromboplastic activity is not markedly affected by heating for 5 minutes in a boiling water bath. This holds true, however, only in tests carried out with fresh oxalated human plasma. When the experiment is performed with plasma stored for some days in a refrigerator, the coagulation time in the presence of heated thromboplastin is greatly increased in comparison to that found with an unheated preparation (Table I).

The thromboplastic preparation from chicken brain is thermostable in the sense that it withstands heating in a boiling water bath for 5 minutes, but it differs from thromboplastin from placenta in some other respects. Preparations of thromboplastin from placenta

[4] Chargaff, E., Moore, G. H., and Bendich, A., *J. Biol. Chem.*, 1942, **145**, 593.

[5] Chargaff, E., *J. Biol. Chem.*, 1944, **155**, 387.

[6] Chargaff, E., Bendich, A., and Cohen, S., *J. Biol. Chem.*, 1944, **156**, 161.

[7] Cohen, S., and Chargaff, E., *J. Biol. Chem.*, 1940, **136**, 243.

[8] Cohen, S., and Chargaff, E., *J. Biol. Chem.*, 1941, **140**, 689.

† Chicken or rooster plasma may be kept liquid without addition of a decalcifying agent if drawn carefully and stored in a clean container in a refrigerator.

TABLE I.
Effect of Heated and Unheated Thromboplastin on Coagulation Time of Fresh and Stored Plasma.

Age of plasma in days	Coagulation time in sec.	
	Unheated thromboplastin	Heated thromboplastin
0	12	12
0	13	13
0	15	18
2	18	23
3	21	28
7	42	185
28	70	> 10 min.

were affected very slightly or not at all by heating. The activity of the heated preparation remained constant when the preparation was kept in a refrigerator. The chicken brain preparations, on the other hand, were affected much more strongly by heating. The coagulation time of heated brain preparation was in some cases about twice that of the unboiled preparation. If, however, a heated preparation was kept in the cold for some days and then tested, its original thromboplastic power was found to be completely restored (Table II).

TABLE II.
Reactivation of Heated Thromboplastin from Chicken Brain Kept at 4-6°C.

Time between heating of thromboplastin and test performed	Coagulation time in sec.	
	Unheated thromboplastin	Heated thromboplastin
Immediately	28	52
1 day	28	30
7 days	26	28

Enzyme chemistry knows similar cases. If, for example, trypsin is inactivated by heating for a short time, reactivation of the enzyme on cooling is complete.[9]

Native chicken or rooster plasma shows no prolongation of coagulation time after storage in a refrigerator for a considerable period of time (1 month) when tested with boiled and reactivated or unboiled chicken brain thromboplastin (Table III). On the other hand, oxalated human plasma, as stated previously, shows when stored a prolongation of its coagulation time as determined on a boiled preparation of thromboplastin from placenta.

Discussion. This paper shows that the "coagulation time" of stored oxalated plasma is greater in the presence of heated thromboplastin than in the presence of unheated thromboplastin. On the other hand, the "coagulation time" of fresh oxalated recalcified plasma or stored "native plasma" (chicken plasma) is the same for heated and unheated thromboplastin.

TABLE III.
Effect of Heated and Unheated Thromboplastin from Chicken Brain on Stored Chicken Plasma.

Age of plasma, days	Coagulation time in sec.	
	Unheated thromboplastin	Heated thromboplastin
0	30	31
1	29	31
3	31	30
30	29	32

An explanation of this phenomenon is suggested by the findings of Quick.[10] Quick believes that prothrombin is composed of two components A and B, which are bound by calcium and thus protected. In decalcified (oxalated) plasma the component A undergoes destruction and gradually disappears from the plasma. "Native" plasma, however does not change in its prothrombin content on storage as it is protected by calcium.

In view of these and our own findings, we assume that thromboplastin too is composed of two components, I and II. One of these is thermostable (I) and withstands heating for 5 minutes, while the other (II) undergoes destruction. The thermostable component (I) acts on plasma in which the prothrombin complex (parts A and B) is still intact, *e.g.*, on fresh oxalated plasma or on "native" plasma (chicken plasma), but does not act on plasma in which the component A of prothrombin is destroyed (stored oxalated plasma). The two components (I and II) of thromboplastin together, *i.e.*, in an unheated thromboplastic preparation, exhibit activity towards stored oxalated plasma. In

[9] Sizer, I. W., *Advances in Enzymology*, 1943, Vol. III, 35-60.

[10] Quick, A. J., *Am. J. Physiol.*, 1943, **140**, 212.

other words, they activate the component B of prothrombin.

It may be concluded, therefore, that the thermostable part of thromboplastin (I) is responsible for the activation of component A of prothrombin. The component B may be activated either by the two parts of thromboplastin together, or by the thermolabile one alone, but the mechanism of activation of component B has not yet been ascertained.

The observation of certain workers that thromboplastin is definitely destroyed by heat may be explained by the fact that the behavior towards heat depends on the method of the preparation. It seems possible that inactivation is due to presence together with the thromboplastic factor of proteins which by denaturation eliminate the active agent.

Since our thromboplastin preparation is thermostable in respect of its ability to coagulate fresh plasma or blood, it is particularly well suited for diagnostic and therapeutic use. It has been used by us for the following purposes: (1) To determine prothrombin time. The aqueous solutions are set up in ampoules for use as necessary; (2) To obtain local stoppage of blood flow; (3) To fix skin transplants. The titrated plasma is placed over the wound together with thromboplastin and calcium and the clot which forms within a few seconds serves as an adhesive for the skin graft (Sano[11]). The advantages of our preparation are its availability in an ampoule, and the fact that it contains human protein. (The undesirable possible by-effects of the use of non-human protein are thus eliminated.) Our clinical experience of this technic will be discussed jointly with Prof. Mandl elsewhere.

[11] Sano, M. E., *Am. J. Surg.*, 1943, **61**, 105.

(4) For injection into man. Since the preparation contains homologous protein undesirable reactions are avoided. Experiments on this aspect are under way.

A sterile and thermostable thromboplastin from chicken tissue may prove useful in plasma culture. The preparation from chicken brain elicits coagulation of chicken plasma within a few seconds. The thromboplastin can be heated and in this way accompanying growth-promoting substances can be destroyed. The coagulation of plasma in tissue cultures is usually induced by the addition of fresh chicken embryo extract which may contain growth-promoting substances to the culture medium. Research on the effect of growth-promoting substances in tissue cultures has been complicated by this circumstance.

Summary. A method of preparation of thermostable thromboplastin from human placenta and of thermolabile but reactivable thromboplastin from chicken brain is described. The properties and differences of these preparations are discussed. Human placenta thromboplastin is not inactivated by heating at 98°C for 5 minutes. Chicken brain thromboplastin is inactivated by the heat treatment but is subsequently reactivated when it is stored at 4-6°C. The different activities of heated and unheated placenta and chicken brain thromboplastin on stored oxalated human plasma and native chicken plasma are explained by the assumption that thromboplastin is composed of two factors, one of them thermostable and the second thermolabile. Clinical, surgical, and research applications of thermostable preparations of thromboplastin are discussed.