formed directly from the glucose, or represents the binding of the soluble form to proteins or some other molecule which is insoluble in cold TCA has yet to be determined.

The dog heart homogenate did not synthesize either glycogen fraction under the same conditions in which the rat heart showed glycogenic activity. Neither form of glycogen of rat heart was synthesized in Krebs Ringer's, though adequate glucose was present, nor in 5.4% glucose when oxygen tension was reduced to 21.5 mm Hg.

Glycogen synthesis was well correlated with oxygen consumption of the tissue homogenates. In the one medium (oxygenated 5.4% glucose) which promoted glycogenesis, oxygen consumption was 303 μl-O_2 per g of wet tissue per hour compared to the utilization of 145 μl-O_2 per g of wet tissue per hour in the unoxygenated sugar medium and the 131 μl-O_2 per g of wet tissue per hour in the oxygenated Krebs Ringer's. The dog heart suspension exhibited the lowest oxygen uptake, using only 114 μl-O_2 per g of wet tissue per hour.

Summary. Glycolysis reduces both glycogen fractions of the rat heart at about the same rate for the first 5 minutes, regardless of the medium used for suspension of the tissue. In Krebs Phosphate Ringer's solution and in unoxygenated 5.4% glucose, the 2 glycogens drop to a low, but stable level. The glycogen synthesized in the sugar medium is primarily the TCA soluble glycogen. The TCA soluble glycogen begins synthesis at the end of 22.5 minutes and synthesis of the TCA insoluble fraction begins 50 minutes later. A distinct species difference was observed in the metabolism of cardiac glycogen in the rat and dog, inasmuch as the TCA insoluble fraction was relatively more stable in the dog.

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Clotting Factor X. Physiologic and Physico-Chemical Properties. (21927)

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The use of serum from various pathological conditions in the thromboplastin generation test(1,2) led to the conclusion that besides the serum factors already known (Factor VII = SPCA, proconvertin and Factor IX = Christmas factor, PTC)* another clotting factor exists in serum for which the designation Factor X(3-5) was proposed. Matching experiments with serum from hemophilia B (deficient in Factor IX) proved to be especially demonstrative.

Method. A modification of the thromboplastin generation test developed in our laboratory was employed(2). The 4 components incubated to generate blood thromboplastin are: Platelet suspension prepared according to Flückiger *et al.*(6). calcium chloride, oxalated plasma adsorbed on barium sulfate (source of antihemophilic globulin) and serum. The first 3 components are kept

* The following numbering of clotting factors is proposed: Fibrinogen (F.I), Prothrombin (F.II), Thromboplastin (F.III), Calcium (F.IV), Labile factor, Proaccelerin (F.V & VI), SPCA Proconvertin (F.VII), antihemophilic Globulin (F.VIII), P.T.C., Christmas Factor (F.IX).

constant (normal platelets and oxalated plasma being used), whereas serum is varied. The results of the test are given in incubation and clotting-times directly measured (not in thromboplastin-units). To study the mixture of 2 sera, both are diluted 1/10 with buffer, then 0.15 cc of each dilution added to the mixture of platelet suspension, adsorbed plasma and $CaCl_2$. *Quantitative determination of Factor VII*: according to Koller, Loeliger and Duckert(7). *Chloroform extract of human brain thromboplastin.* Acetone dried brain powder (1 g) is suspended in chloroform at room temp. 20 min. then filtered and washed with chloroform, (final vol. 50 cc). After evaporation of solvent at 50°C the residue is suspended in 50 cc physiological saline and stored at 30°C. This stock solution is diluted 1/100 with veronal-acetate buffer before use in the thromboplastin generation test, replacing the thrombocytes.

Purification of Factor IX and X. All operations at room temperature. *a*). *Purification of Factor IX by chromatography.* To prepare the column, 1 g of barium sulfate and 1 g of hyflosupercel are suspended in physiological saline, pH 7, and introduced into the column. Excess saline is discarded. Normal citrated serum (1.8 cc serum + 0.2 cc trisodium citrate 0.2 M) is poured on top of column. After the serum 0.5 cc saline is added and elution performed by means of trisodium citrate, 0.14 M, pH 7.8. Flowing speed is slow (1 drop/25 sec.). The eluate is fractionated into portions of 1 cc. The first fractions are rich in Factor VII. Factor IX appears in the fourth fraction. By chromatography Factor IX is obtained free of Factor X which is not eluted by this method. The fraction contains however 5 to 35% of Factor VII. *b*). *Purification of Factor IX by adsorption.* 10 cc of old normal serum (stored at room temperature a few days in order to remove Factor X) and 0.8 cc trisodium citrate 0.2 M (final concentration 0.0148 M) are mixed with 600 mg barium sulfate and stirred 5 min. with a glass rod. After centrifugation, the barium sulfate is washed with 2 cc distilled water and the supernatant discarded. Elution: the barium sulfate is suspended in 1 cc of trisodium citrate 0.14 M, pH 7.8, stirred 5 min. and centrifuged. The supernatant solution contains Factor IX, traces of Factor VII and is Factor X-free. *c*). *Purification of Factor X by adsorption.* 4.5 cc normal serum + 0.5 cc trisodium citrate 0.2 M (final concentration 0.02 M) are mixed with 250 mg barium sulfate and stirred 5 min. After centrifugation the supernatant is discarded, barium sulfate washed with 2 cc distilled water and the solution discarded. Elution: barium sulfate is suspended in 1 cc of trisodium citrate 0.14 M and stirred 3 min. The solution contains Factor X, traces of Factor VII and is Factor IX-free. To exclude any contamination with Factor IX this preparation may be made with hemophilia B-serum.

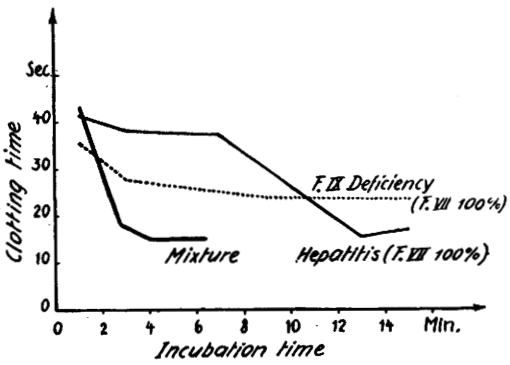

FIG. 1. Normalisation of thromboplastin formation with a mixture of hemophilia B-Serum (F. IX-deficiency) and hepatitis-serum.

Results. Two types of abnormal thromboplastin generation curves. (Fig. 1) Normal serum yields a curve with minimal clotting time of 11 to 12 seconds after incubation time of 3 to 8 minutes. Serum from hemophilia B (deficient in Factor IX) gives a markedly prolonged minimal clotting time, reached however after a normal or almost normal incubation time. On the other hand serum from a patient treated with 3-(1-phenyl-propyl)-4-hydroxycoumarin (= Marcoumar), or from a patient with hepatitis (Fig. 1) produces a greatly delayed curve (incubation time over 12 minutes), but the clotting time finally reached is within normal limits. This curve corresponds to Factor X-deficiency. The difference in shape of the curve is even more pronounced when instead of platelet suspension a

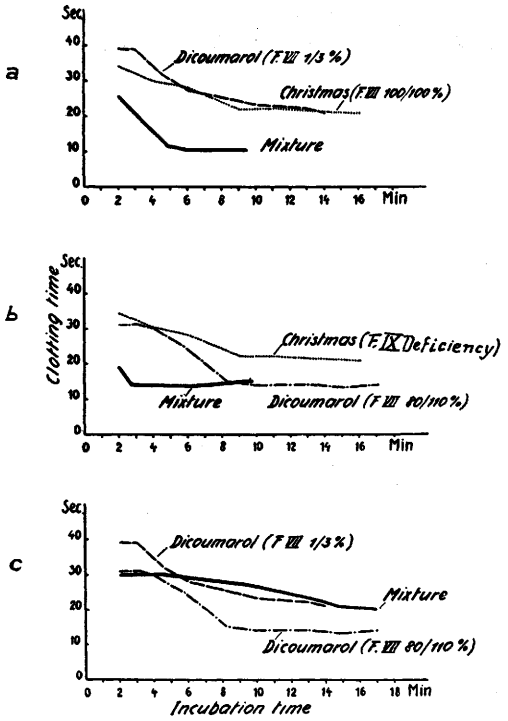

FIG. 2. Effect of a dicumarol derivative (Marcoumar) on thromboplastin formation. (Factor VII activity is given in plasma and serum.) In Exp. C, the concentration of Factor IX is even higher in the mixture than in assay a and b (in Christmas-serum Factor IX is very low), and the concentration of Factor VII is the same as in assay a. Nevertheless thromboplastin formation of the mixture is very poor in c but normal in a and b. A 3rd serum factor has to be assumed.

chloroform extract of brain is used in the generation test. If the concentration of Factor X is very low, thromboplastin formation is almost impossible. Therefore the clotting time (reciprocal to amount of thromboplastin formed) remains high as in Factor IX-deficiency, in these extreme cases(13).

Differentiation of Factor X from Factor VII and IX by matching experiments. A mixture of equal parts of the above mentioned Marcoumar-serum and hemophilia B-serum yields an entirely normal thromboplastin generation. (Fig. 2a). This finding might be in agreement with the hypothesis that insufficient thromboplastin generation of Marcoumar-serum is due to Factor VII-deficiency while that of hemophilia B-serum is caused by lack of Factor IX. However the following observation contradicts this view: Serum of a patient who had first been given Marcoumar, then, because of bleeding, vit. K_1, and whose Factor VII concentration had returned to normal (100% in plasma and in serum) produced a markedly delayed thromboplastin generation. Hemophilia B-serum normalises this clotting defect. (Fig. 2b). Therefore lack of Factor IX cannot account for the delay of thromboplastin generation, nor can Factor VII-deficiency do so. A third serum-factor (Factor X) must be involved. If the 2 Marcoumar sera (with 10 resp. 100% Factor VII) are mixed, there is no normalisation of thromboplastin generation. (Fig. 2c) Serum of epidemic hepatitis, of hepatic cirrhosis, of the new-born behaves in the same way as Marcoumar-serum. (Table I). Stored serum is also abnormal in thromboplastin generation although the concentration of Factor VII and IX remain unchanged during several days at room temperature. After 8 days of storage Factor VII 160% and Factor IX were normal. The delayed thromboplastin formation is therefore due to deficiency of another serum factor.

Purified Factor IX, mixed with hemophilia B-serum produces a normal thromboplastin generation; the same is true with purified Factor X when mixed with Marcoumar-serum. Matching of both purified preparations (Factor IX and X) gives a normal thromboplastin generation in spite of the very low Factor VII content of the mixture. The chromatographic fraction containing no factor X produces a very delayed thromboplastin generation when mixed with Marcoumar-serum.

Objections to these matching experiments. Hemophilia B-serum used in most matching experiments (Table I) contains considerable amounts of prothrombin, as a consequence of the reduced prothrombin consumption. During the thromboplastin generation test prothrombin is converted into thrombin and therefore may influence the results. Isenschmid(9), using fibrinogen as substrate instead of calcium-free plasma, was able to measure the amount of thrombin formed simultaneously with thromboplastin generation. This amount was substracted from the total quantity of thrombin measured with calcium-free plasma as substrate. In this way a "cor-

TABLE I. Matching Experiments.*

No.	Serum I	F. VII	IX	X	Serum II	F. VII	IX	X	Mixture† F. VII	IX	X	Thromboplastin generation	
		%				%			%				
1.	Marcoumar	3	+	—	Hemophilia B	100	—	+	50	+	+	normal	Fig. 2a
2.	Marcoumar + vit. K1	110	+	—	"	100	—	+	105	+	+	"	Fig. 2b
3.	Hepatitis, mild	100	+	—	"	100	—	+	100	+	+	"	Fig. 3
4.	*Idem*	100	+	—	Marcoumar	40	+	—	70	+	—	delayed	
5.	Hepatic cirrhosis	75	+	—	Hemophilia B	100	—	+	90	+	+	normal	
6.	New-born	45	+	—	"	100	—	+	75	+	+	"	
7.	Stored normal serum (8 days)	160	+	—	"	100	—	+	130	+	+	"	
8.	Factor IX purif. chromat.	35	+	—	"	100	—	+	70	+	+	"	
9.	*Idem*	35	+	—	Marcoumar	25	+	—	30	+	—	delayed	
10.	Factor IX purif. adsorpt.	7	+	—	Hemophilia B	100	—	+	55	+	+	normal	
11.	*Idem*	7	+	—	Marcoumar	30	+	—	20	+	—	delayed	
12.	Factor X purif. adsorpt.	7	—	+	"	35	+	—	20	+	+	normal	
13.	Factor X purif.	7	—	+	Hemophilia B	100	—	+	55	—	+	abnormal	
14.	Factor X purif. adsorpt.	7	—	+	Factor IX purif. adsorpt.	7	+	—	7	+	+	normal	

* All sera I and II used alone, produce an abnormal thromboplastin generation; Factor IX and X can be determined only approximately; + means normal, — means diminished or lacking activity in thromboplastin generation test; "purif. chromat." means purified by chromatography; "purif. adsorpt." means purified by adsorption.

† In a mixture of 2 sera resulting in normalisation of thromboplastin generation Factor IX and X concentrations do not exceed 50% of normal values.

As example of normalisation see Fig. 1, 2a and b. As example of non-normalisation see Fig. 2c.

rected" curve was obtained, which showed that normalisation of thromboplastin generation, by mixing Marcoumar- and hemophilia B-serum, persists. Only the "over-normalisation" has to be attributed to the prothrombin content of hemophilia B-serum. Isenschmid could demonstrate that thrombin has no accelerating effect on thromboplastin formation; therefore simple substraction of its effect is allowed. Another objection is furnished by the observation that matching of Marcoumar- and hemophilia B-serum does not always produce a complete normalisation of thromboplastin generation. In fact Dicoumarol and its derivatives although they lower primarily Factor X (besides prothrombin and Factor VII), affect also, to a lesser degree, Factor IX. Marcoumar, (the Dicoumarol-derivative we used most), lowers first the concentration of Factor X alone, and after a week or 2 the activity of Factor IX. Bis-3, 3'-(4-hydroxycoumarinyl) - ethyl - acetate (tromexan), on the other hand, seems to lower Factor IX more rapidly. It is evident that hemophilia B-serum cannot normalise the clotting defect of a serum simultaneously deficient in Factor IX and X. It has been suggested that a factor-X deficiency is a slight deficiency of Factor IX. This opinion is in contradiction to the results obtained by dilution of normal serum(2) where both incubation and coagulation time are prolonged, not incubation time alone. The assays with purified factors (Table I, No. 8-14) also contradicts this opinion as well as the experiments illustrated in Fig. 2. Another objection was that an excess of *antihemophilic globulin* might explain normalisation produced by mixing hemophilia B-serum with various pathological sera (Table I). We increased the concentration of antihemophilic globulin in the incubation mixture 5 times (by using a 1:5 instead of a 1:25 dilution of $BaSO_4$-plasma) without obtaining a normalisation of throm-

boplastin generation with hemophilia B or Marcoumar-serum. It is evident that excess of one clotting factor can compensate *partially* for the deficiency of another factor. A complete normalisation however has never been achieved by this procedure.

Insignificant role of Factor VII in blood thromboplastin formation. In Table I it may be seen (No. 14) that thromboplastin generation of the mixture is normal with as low a Factor VII content as 7% (100% Factor VII = normal concentration in plasma) whereas it is delayed with a concentration of 70% (No. 4 of Table I). A rise of Factor VII from 10 to 110% under the influence of Vit. K does not change significantly the delayed thromboplastin formation (Serum I in No. 1 & 2 of Table I). On the other hand a decrease of Factor VII in serum from 150% to 18% in the beginning of Marcoumar-treatment did not delay thromboplastin formation. It may therefore be concluded that Factor VII has very little, if any, influence in blood thromboplastin generation. (See also Fig. 2).

Physiological properties of Factor X compared with those of Factor IX. Incubation mixtures are prepared with constant amounts of all factors necessary for thromboplastin formation except Factor X, the concentration of which is varied from 1 to 100%. As seen in Fig. 3 the speed of thromboplastin formation is greatly enhanced by increasing amounts of Factor X, whereas the *amount* of thromboplastin produced (*i.e.* the clotting time finally reached) is practically unchanged. If in the incubation mixture the concentration of Factor IX is varied while all other factors are kept constant, the clotting time (corresponding to amount of thromboplastin formed) is primarily influenced (Fig. 4), whereas incubation time differs only slightly. It seems therefore that Factor IX is a precursor of blood thromboplastin and that Factor X has to be considered as an *accelerator of blood thromboplastin formation.* There is another difference between the 2 factors: in Factor IX-deficiency prothrombin consumption is markedly reduced whereas in "pure" Factor X-deficiency it is normal.

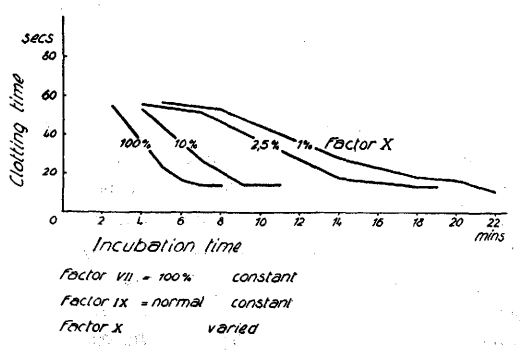

FIG. 3. Influence of variable Factor X concentrations, all other factors being kept constant. Clotting time normal, incubation time prolonged.

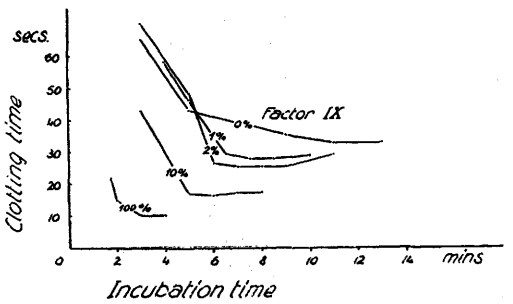

FIG. 4. Influence of variable Factor IX concentrations, all other factors being kept constant. Incubation time in normal range, clotting time prolonged.

Physico-chemical properties of Factor X. The properties of Factor X are almost identical with those of serum factors VII and IX. However its resistance to storage is lower. It disappears from normal serum in a few hours or a few days at room temperature. It is denatured at 50°C. Its affinity to $BaSO_4$ is more pronounced than that of Factor VII and IX (see method of purification). By chromatography it is irreversibly adsorbed. It is also adsorbed by $BaCO_3$ and $Ca_3(PO_4)_2$. In diluted solution (serum 1/10 with veronal acetate buffer pH 7.35) it is moderately activated at room temperature within 20 to 30 min.

Factor X compared to other recently described clotting factors. Factor X is not identical with PTA(10), the latter being only slightly adsorbed on barium sulfate. Deficiency of PTA produces an abnormal thromboplastin generation only if both $BaSO_4$ plasma and serum of the patient are used in the test. PTA is therefore neither adsorbed on $BaSO_4$ nor consumed during coagulation.

Two new clotting factors described by Spaet(11) and Ratnoff(12) are not identical with factor X because they are not adsorbed on barium sulfate.

Summary. 1. A new clotting factor, factor X, participating in blood thromboplastin formation is demonstrated by matching experiments with various pathological sera. The possible influence of thrombin and factor VII is eliminated. The objection that factor X deficiency may be nothing else than a slight deficiency of factor IX is discussed. This hypothesis is in contradiction with our results. 2. Factor X is present in normal serum and in hemophilia B-Serum, absent in hepatitis-serum and in serum of Marcoumar-(Dicoumarol derivative) treated patients. The affinity of factor X to barium sulfate is greater than that of Factor VII and IX. This property is utilised for the purification of Factor X. 3. Factor X is not identical with PTA and the new factors of Spaet *et al.* and of Ratnoff *et al.*

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Response of Visual Area to Callosal Impulses in the Cat.* (21928)

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There are still uncertainties concerning the collosal connections of the visual area. The anatomical(1-3) and the oscillographic data are equivocal. Reactions of the area to a synchronized volley of callosal impulses have been found in the cat by Curtis(3) and also (from his experimental map) by Garol(4). But Curtis(3), McCulloch and Garol(5), Bailey, Bonin and McCulloch(6) were unable to discover any commissural connections from area 17 in the cat, the monkey and in the chimpanzee.

In the unanesthetized cat ("encéphale isolé" préparation) callosal responses of the visual area, which can be recorded with great regularity, have revealed special features which seem of interest.

Methods. All observations have been done on adult cats after an initial ether narcosis during which the spinal cord had been cut at the C_1 level, the optic nerve prepared on one side and the cortex exposed on both sides. The callosal volley was produced by a thyratron shock of 0.5 millisecond duration applied to a point of the contralateral gyrus lateralis symmetrical to the point whose potentials were recorded. The responses of various other areas were displayed simultaneously on the second beam of the cathode ray oscilloscope. The differential amplifier systems had an overall time-constant of 0.1 sec. The cortical stimulation was bipolar, the recording of the potentials monopolar. The response of the visual area I to shocks on the contra-

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