best to supply total nutriment parenterally. Part of the problem involves how best to combine amino acids, minerals and calories in the relatively small volumes of liquid which can be given parenterally. At the present stage of development it is generally possible to provide only those nutrients for recuperation to the point where full oral intake is resumed. Potassium appears to fit into this special group of nutrients.

Summary. 1. Results of this study point to the critical role of potassium in amino acid utilization following protein depletion. Rats depleted only of protein failed completely, even when offered a complete source of amino acids, if at the same time potassium was withheld. If phosphorus was withheld during the repletion period, the rate of recovery was lessened, but complete failure and deaths did not result as with potassium deprivation. Deprivation of sodium, calcium or magnesium did not appear to have immediate limiting effects on the appetite of protein depleted rats for a complete amino acid source, or on their ability to recover lost weight. 2. Fourteen mg of potassium per rat day appeared to meet the requirement for this element. Addition of 10 mQv of potassium per liter to an intravenous protein hydrolysate appears to supply an effective, safe ratio of potassium to amino acid nitrogen.

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Isolation and Purification of Plasma Thromboplastin: Its Physico-Chemical and Biologic Properties.* (20279)

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In the course of studies on the isolation of various platelet and plasma factors active in the process of blood coagulation the observation was made that prothrombin and stable factor cannot be adsorbed on $BaSO_4$ from citrated plasma(1,2). A saline eluate from the adsorbent, however, contained a factor able to shorten considerably the clotting time of recalcified human plasma and increase markedly the prothrombin utilization of native platelet-poor human plasma. This article describes some of the properties and the biologic activity of this factor which is tentatively identified as "plasma thromboplastin" (PT).

Materials and methods. (a) *Separation of PT from other coagulation factors.* Blood was collected from healthy individuals in Silicone† coated syringes and through Arquad 2-C‡ coated 19-gauge needles using one-tenth volume sodium citrate 0.2 M as the anticoagulant. Platelet-rich plasma was separated

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† General Electric Dri-Film 9987.

‡ Available from Armour Co., North Chicago, Ill.

by centrifugation at 1,000 rpm for 15 minutes at 4°C and transferred to glass test tubes. These were kept for 30 minutes at 37°C and then centrifuged at 4,000 rpm for 30 minutes at 4°C to remove the remaining formed elements. The supernatant was adsorbed with $BaSO_4$ (analytical grade) (50 mg/ml). Prothrombin, fibrinogen, labile factor (proaccelerin, accelerin, PPCF, plasma ac-globulin) and stable factor (proconvertin, convertin, SPCA, factor VII) were found in practically unmodified concentration and activity in the supernatant plasma. The $BaSO_4$ was washed thoroughly twice with distilled water at 4°C; finally an eluate was obtained by treatment with 0.85% NaCl solution (or sodium citrate 0.2 M) equal in volume to 1/10 that of the original plasma. The eluate was free of known coagulation factors and contained a highly potent preparation of PT. Optimum elution was obtained at pH 6.3 and 37°C. Maximum activity was obtained after 15 to 20 minutes incubation in glass. (b) *Further purification of PT.* The eluate containing PT was again adsorbed on $BaSO_4$ and the PT eluted with 0.85% NaCl solution. After acidification to pH 5.0 with bubbling CO_2 at 5°C a precipitate formed which was separated by centrifugation and found inactive. The supernatant yielded a white precipitate after 25% saturation with $(NH_4)_2\ SO_4$. The precipitate was redissolved to the original volume of the eluate with 0.85% NaCl solution and dialyzed against 0.85% NaCl at 5°C for 24 hours. The final product represented a highly purified preparation of PT, with an average of 20 units (as defined below) per mg of N. (c) *Assay of PT.* The assay of PT activity was carried out as follows: Blood was collected in Silicone-coated glassware with the double-syringe technic(3) through Arquad 2-C coated needles without anticoagulant. It was centrifuged immediately at 4,000 rpm for 30 minutes. The "native" plasma contained approximately one to 2 platelets/cu mm and would only clot after several hours when incubated at 37°C in Silicone-coated test tubes. The prothrombin activity of its serum, as determined with a one-stage procedure(4) would be as high or higher than that of the original plasma. 0.1 ml of the PT eluate obtained as described in (a) was added to 0.9 ml of platelet-poor native plasma in glass test tubes. Clotting occurred promptly. One hour later the prothrombin activity of the serum was determined. The prothrombin time obtained was translated into percentage of prothrombin activity by the use of a dilution curve. This was prepared by the following technic. A potent preparation of PT eluate was diluted from 100 to 10% with 0.85% NaCl solution. 0.1 ml of each dilution was added to 0.9 ml of platelet-poor native human plasma. The prothrombin time of the serum from each mixture was then obtained one hour after clotting. The actual times were then plotted against dilution on semi-logarithmic graph paper. Activity of PT was calculated as the difference between 100 and the per cent activity of the serum. Thus, if serum exhibited a prothrombin activity of 40% the potency of the PT added to the native plasma would be (100–40) 60%. (d) *Unit of PT.* With the availability of a purified preparation, a unit of PT was tentatively established. This is "the amount of material which added in the volume of 0.1 ml to 0.9 ml of platelet-poor native human plasma will halve the prothrombin activity of the resulting serum one hour after the completion of clotting as measured by the one-stage prothrombin consumption test." As already stated, most of the purified preparations contained approximately 20 units of PT per mg of N.

Results. (a) *Activity of PT.* As indicated in Table I, PT was able to shorten markedly the clotting time of native plasma and of fresh normal human recalcified oxalated plasma. It normalized the clotting time, if prolonged, and the prothrombin utilization during clotting of native normal platelet-poor plasma (collected in Silicone) and plasma of patients with known hemophilia, thrombocytopenic purpura, and thrombasthenia. On the other hand, PT was unable to correct the delayed prothrombin time of plasma from patients treated with high doses of Dicumarol (showing marked deficiency of prothrombin and stable factor). Also, the delayed prothrombin time of stored human plasma (depleted of labile factor) was not affected. These results were confirmed by experiments in which PT

TABLE I. Activity of Plasma Thromboplastin.*

	Clotting time, min.		Prothrombin activity of serum, %	
	Before adding PT	After adding PT	Before adding PT	After adding PT
1. Native human plasma	5'15"	2'30"	12.5	0
2. " platelet-free human plasma	90'	7'15"	95	0
3. Normal human oxalated plasma (recalcified)	2'15"	42"	—	—
4. Hemophilic plasma	29'	12'	100	4.5
5. Thrombocytopenic plasma	6'	3'	82	0
6. Plasma from patient with thrombastenia	—	—	58	2
	Prothrombin activity of plasma, %		Labile factor activity of plasma, %	
	Before adding PT	After adding PT	Before adding PT	After adding PT
7. Plasma of Dicumarolized patients	12	11	—	—
8. Stored human plasma	2.5	2.5	10.5	12

* In each instance, PT eluate was added to various plasmas in 1/10 (equivalent to the amount of PT thromboplastin in 1 ml of plasma, since purified preparation is concentrated 10 times). The prothrombin activity of serum was determined 1 hr after completion of clotting. Each instance represents a typical experiment.

TABLE II. Activity of Plasma Thromboplastin from Various Citrated Plasmas, Platelets and Serum.*

	Prothrombin activity of serum, %	Plasma thromboplastin activity, %
1. Normal platelet-rich human plasma	1.5	98.5
2. " " " " " (incubated in Silicone)	75.5	24.5
3. " " -poor " "	82.0	18.0
4. Suspension of washed platelets†	86.0	14.0
5. " " " " + platelet-poor plasma	29.0	71.0
6. " " " , ether treated platelets + platelet-poor plasma	91.5	8.5
7. Normal human serum, shortly after coagulation	91.5	8.5
8. Plasma from heparinized patients	22.0	78.0
9. " " Dicumarolized patients (plasma prothrombin activity 9%)	1.0	99.0
10. Hemophilic plasma	85.0	15.0
11. Thrombocytopenic plasma	80.5	19.5
12. Hemophilic + thrombocytopenic plasma	9.5	90.5

* In each instance 1/10 ml of purified PT obtained from plasmas, platelets or serum as described was added to 9/10 ml of platelet-poor fresh native human plasma; mixture allowed to clot and prothrombin time of serum determined 1 hr after completion of clotting. Each instance represents a typical experiment.

† Platelets were resuspended, after washing, in 1/3 volume saline; ground with $BaSO_4$ and the $BaSO_4$ eluted with saline solution.

eluates were prepared from plasma of various patients (Table II). Fully active eluates were obtained not only, as expected, from normal citrated plasma but also from plasma of severely heparinized or Dicumarolized patients. Human plasma stored for one week at 4°C exhibited only 15% labile factor activity but 50% plasma thromboplastin activity. On the other hand, no or minimal PT was obtained from plasma of severe hemophiliacs and patients with thrombocytopenic purpura; from isolated and washed platelets; from platelet-poor and platelet-free plasma, and from platelet-rich plasma carefully collected and kept in Silicone-coated surfaces. When thrombocytopenic and hemophilic plasmas; and isolated platelets and platelet-poor plasma were mixed, an eluate of normal PT activity was obtained. This did not occur when platelets were treated with ether prior to incubation with plasma. Eluates from serum immediately and at progressive intervals after clotting were found to contain practically no PT activity.

TABLE III. Properties of Plasma Thromboplastin.

1. Stable at 56°C for 30 min.
2. Fully active after 1 wk storage at −20°C; 50% active after 1 wk storage at 4°C; practically inactive after 3 wk storage at 4°C.
3. Not dialyzed against 0.85% NaCl solution for 24 hr.
4. Not sedimented after centrifugation at 40000 RPM for 1 hr.
5. Absorption curve shows maximum optical density at 278 μ.
6. Adsorbed on tri-calcium phosphate gel and barium sulfate from citrated plasma; eluted from it by 0.85% NaCl solution or 0.2 molar sodium citrate; maximal elution at pH 6.3, and 37°C.
7. Absorbed on 20%, 30% and 50% asbestos filters.
8. Completely precipitated from solution by 25% saturation with ammonium sulfate.

(b) *Properties of PT* (Table III). Samples of a saline eluate of Plasma Thromboplastin were still fully active after one week of storage at —20°C; samples stored at 4°C showed a loss of 50% activity after one week and total loss after 3 weeks. PT activity was relatively unimpaired after 30 minutes incubation at 56°C and after dialysis against 0.85% NaCl solution for 24 hours. Centrifugation of the eluate at 40,000 rpm for one hour failed to produce any precipitation and the upper layer was fully active. After centrifugation at 40,000 rpm for one hour maximal PT activity was obtained from the uppermost layer of plasma. A purified eluate showed maximum absorption at 278 μ (Fig. 1). Further chemical and electrophoretic data are being obtained. Asbestos filters 20%, 30%, and 50% absorbed PT completely. This, as already mentioned, could be precipitated from solution by 25% saturation with $(NH_4)_2SO_4$.

(c) *In vivo effects of PT.* PT was administered to 4 patients with thromboplastin-deficiency states, 3 with "amegakaryocytic" thrombocytopenic purpura and one with severe hemophilia. PT from 4 pints of freshly collected blood was prepared as described in the section on methods (a) and injected intravenously in a total volume of approximately 150 ml in 0.85% NaCl solution over a period of 25 minutes. There was no change in pulse rate, body temperature or blood pressure, nor did the patients complain of any subjective symptoms. In one of the patients receiving 2 injections at 15 days interval there was, likewise, no anaphylactoid reaction to the second administration.

In the 3 thrombocytopenic patients the prothrombin utilization during clotting was significantly increased for at least 8 hours following the injection (Fig. 2) and the clotting time of recalcified plasma slightly shortened, while no important change in the con-

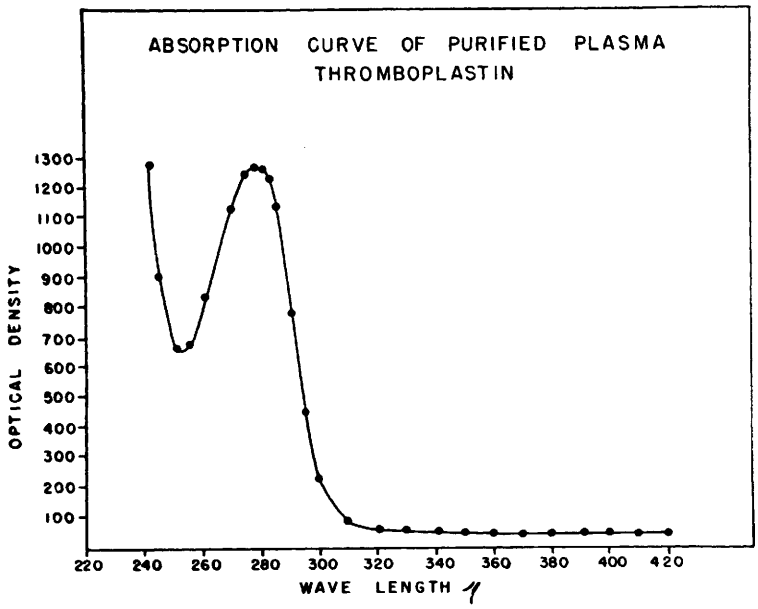

FIG. 1. Absorption curve of purified plasma thromboplastin.

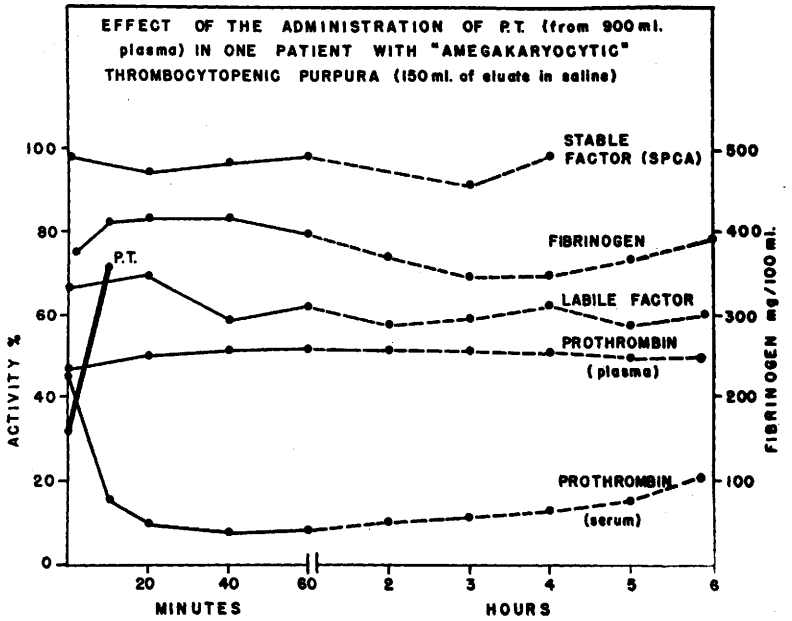

FIG. 2. Effect of administration of "plasma thromboplastin" (from 900 ml of plasma) in one patient with "Amegakaryocytic" thrombocytopenic purpura (150 ml of eluate in saline.

centration or activity of plasma prothrombin, labile factor, stable factor or fibrinogen was noted. The prolonged bleeding time and positive tourniquet test were unaffected. The patient with hemophilia showed only minor and transient acceleration in the clotting time and increase in the prothrombin utilization immediately after the administration of PT. The patient, however, had received only 1/3 of the minimum amount of PT *in vivo* needed to correct his hemorrhagic defect *in vitro* and therefore, possibly, an insufficient amount to cause any changes. The platelet count was halved immediately after infusion but returned to the original level within one hour.

Discussion. Physico-chemical properties and biologic activity differentiate PT from other known coagulation factors. The development of this agent requires the presence of at least one or more platelet and plasma factors and of a foreign surface. This makes it unlikely that PT represents an independent coagulation factor. It is generally conceded that the first stage of blood coagulation consists in the activation of thromboplastin following shedding of blood. It is likely that PT represents the product of this preliminary phase of blood clotting. Calcium is not apparently needed for the evolution of PT which, however, requires the presence of a foreign surface. Whether such a surface activates a plasma component or causes the liberation of a platelet factor or both cannot be stated from the data available at this time.

Summary. 1. A factor (plasma thromboplastin) can be absorbed from citrated human plasma which shortens the clotting time of normal oxalated recalcified human plasma and increases the prothrombin utilization of platelet-poor hemophilic and thrombocytopenic plasma. This factor has been eluted and purified. An assay for its activity has been developed. A unit of activity, physico-chemical properties and biologic activity have been defined. 2. Plasma thromboplastin can be clearly differentiated from other known coagulation factors on the basis of its properties; its evolution requires one or more platelet and plasma factors and contact with a foreign surface. It probably represents the final product of the first phase of blood coagulation (activation of thromboplastin). Its administration has been proven to correct *in vivo* the defective prothrombin utilization

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Effect of Feeding Fat on Duration of Thiopental Anesthesia. (20280)

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Studies of the mechanism of thiopental detoxication have led to the conclusion that its short duration of action is due to its localization in the fat depots of the body, rather than to its rapid destruction(1). This conclusion implies that the amount of body fat greatly influences the duration of thiopental anesthesia. Hermann and Wood(2) successfully demonstrated this by showing that a 5 g % decrease in body fat resulted in a 100% increase in the duration of thiopental anesthesia. As a result of these findings it was deemed of interest to determine the effect of dietary fat on the duration of thiopental anesthesia.

Methods. A homogeneous group of non-starved albino white rats was divided into two groups with an equal number of both sexes in each group. Two hours before the injection of thiopental each member of one group was fed 2 cc of corn oil by stomach tube while the other group received nothing, other than their normal diet. Then 30 mg/kg of sodium thiopental* (10 mg/cc in isotonic saline) was injected into a tail vein. The anesthesia time was determined by the duration of the loss of the righting reflex, that is the time taken for the animals to roll over onto their feet from the supine position. The rats were not injected oftener than once every 14 days to avoid an acute thiopental tolerance (3).

Results. Throughout this experiment considerable difficulty was encountered because of a large day to day variation in anesthesia time, which cannot be satisfactorily explained. On some days a mean sleeping time of 76 minutes for the control group and 25 minutes for the oil group has been obtained; while on other days, using the same dose, a mean sleeping time of 22 minutes for the control group was obtained as opposed to 11 minutes for the oil group. The results tabulated in Table I Exp. A and B demonstrate this wide day-to-day variation, but also show that in spite of this daily variation there consistently remained a significant difference between the sleeping time of the control and the oil fed group. The data presented in Table I, Exp. C were obtained by processing the entire lot of 34 rats in one day to eliminate the day-to-day variation. It can be readily observed that a highly significant decrease in anesthesia time was induced by the oral administration of oil.

Discussion. A review of the work of Brodie *et al.*(4) suggests a possible explanation for this difference. Brodie has shown that following the administration of thiopental a shift in concentration occurs until an equilibrium is reached at which time the body fat contains about 15 times the concentration of thiopental of the plasma and other body tissues. Under the conditions of this experiment a chylomicronemia and hyperlipemia might be expected to occur following the ingestion of the oil. When the thiopental is injected it may be taken up by the chylomicrons, thus reducing its effective blood concentration and therefore decreasing its anesthetic action. This might be visualized as a partition of the thiopental between the lipid and aqueous phases of the body. The additional lipid supplied by the ingestion of the oil causing a reduction in the thiopental in the aqueous phase.

* Pentothal (Abbott).