TABLE I. Coproporphyrin III Extracted from Subdivisions of Pig Central Nervous System.

γ of coproporphyrin III/100 g of tissues*					
Cerebrum	Midbrain	Medulla oblongata	Cerebellum	Spinal cord	Total CNS avg
3.05 ± 0.1	4.23 ± .27	4.85 ± .31	3.10 ± 0	3.16 ± .22	3.68

* Mean ± S.E.

porphyrin occurs in the medulla oblongata (Table I). This is only slightly higher than the amount found in the midbrain. Values for midbrain and medulla oblongata are highest, while values for the cerebrum, cerebellum and spinal cord are slightly lower than the average for the total central nervous system. The average for all 5 subdivisions of the nervous system of the pig is in close agreement (3) with the quantitative determination of coproporphyrin in total rabbit brain (3.56 γ/100 g).

Discussion. Klüver(1) observed the 625 mμ fluorescence band in funiculi of the spinal cord, and other fiber tracts of the central nervous system. Examination revealed that the 625 mμ band was absent in peripheral parts of cranial nerves.

Subsequent investigations have been limited to porphyrin content of total brain. Our results indicate that coproporphyrin III concentration varies at different levels of the central nervous system. This observed variation or gradient in coproporphyrin III concentration with a maximum in the medulla oblongata, lends support to the hypothesis that the central nervous system porphyrins have some functional significance. It should be emphasized that our values represent only the coproporphyrin III content of parts of the central nervous system.

Summary. The central nervous systems of pigs were collected and divided into the various gross anatomical subdivisions. Equal weights of cerebrum, midbrain, medulla oblongata, cerebellum, and spinal cord were extracted for coproporphyrin. A higher concentration of coproporphyrin III was found in the medulla oblongata and brain stem than in cerebrum, cerebellum, or spinal cord. The observed differential coproporphyrin III concentration in various subdivisions of the central nervous system supports the hypothesis that porphyrins in the nervous system may have functional significance.

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Effects of Varying Concentrations of Platelets and Their Lyophilized Derivatives on Generation of Thromboplastin.*† (22944)

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It has previously been reported that high concentrations of platelet material, fresh, stored at −15°C, or lyophilized, inhibit the coagulation of recalcified, platelet-depleted plasma. Adjustment of the concentration of platelet material to approximate the equivalents of physiological platelet levels, brought the recalcification time into the normal range (1). The nature of these phenomena was

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further investigated by examining the effects of varying concentrations of platelet materials in the generation of plasma thromboplastin.

Methods and materials. Fresh and lyophilized platelet concentrates (PC) were prepared as described previously(1,2). Platelet-depleted plasma was prepared by centrifugation at 32,000 g for 30 minutes. Plasma from normal and thrombocytopenic donors was used. A modification of the thromboplastin generation test of Biggs and Douglas(3) was employed. Prothrombin-depleted plasma was prepared by treatment with $BaSO_4$. The $BaSO_4$ suspension (40 mg $BaSO_4$ per cc of plasma) was shaken for 30 minutes and centrifuged at 32,000 g for 30 minutes. The one-stage prothrombin time of this preparation is in excess of 180 seconds. The 0.1 cc aliquots of the incubation mixture were drawn into tuberculin syringes which contained 0.1 cc of a 0.025 M solution of $CaCl_2$. Contents of syringe were then expelled forcefully into the tube containing substrate plasma. The substrate mixture was rotated manually until the first fibrin strands appeared, which was taken as the end-point. Suspensions of platelet material, varying in concentration between approximate equivalents of 5,000 and 20,000,000 platelets/cu mm, were added to the incubation mixture and the generation of thromboplastin was estimated.

Results. Representative data are summarized in Table I and in Figs. 1 and 2 for fresh and lyophilized platelet materials, respectively.

It can be seen from these data that the minimum clotting time and the incubation time, at which the corresponding maximum activity develops, vary in approximately the same manner with analogous concentrations of the 2 types of platelet preparations. At high concentrations the generation of plasma thromboplastin activity is inhibited. Normal values are attained at the approximate equivalent of physiological concentrations.

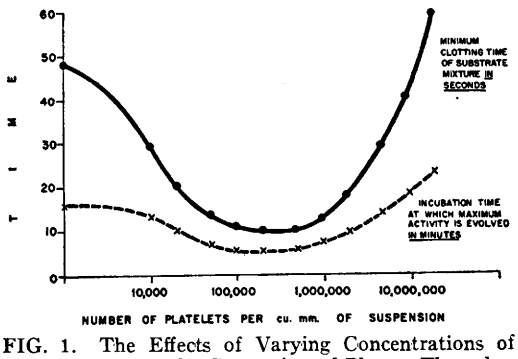

FIG. 1. The Effects of Varying Concentrations of Fresh Platlets on the Generation of Plasma Thromboplastin.

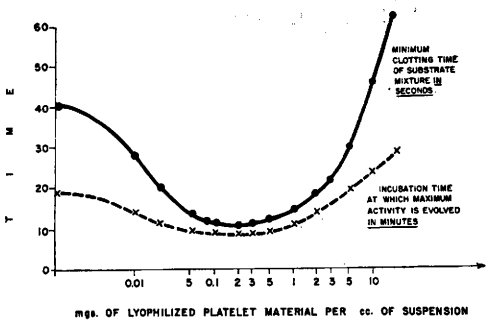

FIG. 2. The Effects of Varying Concentrations of Lyophilized Platelet material on the Generation of Plasma Thromboplastin.

TABLE I. Effects of Varying Concentrations of Platelet Materials on Generation of Plasma Thromboplastin Activity.

No. of platelets or equivalent of suspension	Fresh platelets		Lyophilized platelets	
	Incubation time (min.)*	Min clotting time (sec.)†	Incubation time (min.)*	Min clotting time (sec.)†
× 1000				
20,000	23	59	28	62
10,000	18	40	23	45
5,000	14	29	19	29
2,000	9	18	14	18
1,000	7	13	11	14
500	5	10	9	12
200	5	10	8½	11
100	5	11	8½	11
50	7	14	9	13
20	10	20	11	19
10	13	29	14	28
Control	16	48	19	41

* Time required for incubation mixture to reach maximum thromboplastic activity.

† Clotting time of substrate when incubation mixture has reached maximum thromboplastic activity.

Discussion. It is apparent from the data presented here that generation of plasma thromboplastin activity is related to amount of platelet material in the incubation mixture. The amount of platelet material determines the maximum thromboplastin activity, as

measured by the minimum clotting time of the substrate, and the rate at which that level of activity is developed.

The inhibitory effects of high concentrations of platelet materials on recalcification time(1) are paralleled by inhibition of the generation of plasma thromboplastin. The mechanism of the inhibitory phenomena is obscure, but there are indications that platelets may contain specific activities which inhibit coagulation(4,5,6). Platelet materials do not activate the generation of thromboplastin excessively at any concentration. Extracts(4) and fractions of platelet material behave similarly in regard to inhibition and to the absence of excessive activation of coagulation of varying concentrations of the respective platelet preparations.

These *in vitro* studies, therefore, do not present contraindications to the *in vivo* administration of such materials in regard to the hypothetical hazards of thromboembolic phenomena. In parallel animal and clinical studies here(7,8) and in animal studies by others(9) no evidence for hyper- or hypocoagulation has been obtained after intravenous infusions of large amounts of various types of platelet preparations.

It can be seen that lyophilized platelet material produces approximately the same degree of activation and inhibition as fresh platelets at corresponding concentrations. The lyophilized material, however, requires longer periods of incubation than fresh platelets for reaching maximum levels of thromboplastin.

The effects described here may play a role in the hemorrhagic phenomena encountered in some cases of idiopathic thrombocythemia‡ and in the thrombocythemia secondary to polycythemia, leukemia, the post-splenectomy state and others.

Summary. High concentrations of fresh and lyophilized platelet materials inhibit the generation of plasma thromboplastin. Lyophilized platelet material, at the approximate equivalent of physiological concentrations, produces a normal level of plasma thromboplastic activity. Thromboplastin generation is not activated excessively at any concentration of these preparations.

‡ Personal communication.

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