TABLE I.
Total Lipids, Protein, Water, and Ash.

	Normal mice (7 animals)					Injected mice showing marked wt gains (7 animals)				
	Wt, g	Lipids g	Protein g	Water g	Ash g	Wt, g	Lipids g	Protein g	Water g	Ash g
Mean	22.1	2.42	3.65	16.2	.74	45.6	18.07	5.55	20.0	.72
Stand. error	.9	.33	.12	.7	.05	2.4	1.94	.26	.9	.09

tions of goldthioglucose. Analysis of total body lipids, protein, water, and ash showed these weight gains to be primarily due to an increase in adipose tissue. This obesity occurred in approximately one-third of the survivors of sublethal doses of goldthioglucose. Except for centrolubular fatty infiltration of the liver in obese animals, no anatomic lesions were found in the organs examined.

16972

Effect of Aminophylline on Plasma Prothrombin and Ac-Globulin in Dogs.

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Several conflicting reports have appeared in recent literature concerning the effect of the administration of methylxanthines on prothrombin activity. Field and coworkers[1] reported a hyperprothrombinemia following oral administration of these drugs to experimental animals in doses ranging from 10-400 mg/kg body wt. Scherf and Schlachman[2] also reported a shortening of prothrombin time in human subjects given therapeutic doses of aminophylline and theophylline with sodium acetate either orally or intravenously. However, a number of investigators have been unable to confirm these results. Quick[3] noted no increase in the prothrombin level of dogs and rabbits fed large doses (100-200 mg/kg) of methylxanthines. In a careful study, Holland and Gross[4] reported that methylxanthines, when given to dogs either in single intravenous doses of 25-50 mg/kg, or in daily oral doses of 20 mg/kg, produced no shortening in prothrombin time. Furthermore, single intravenous doses of 0.5 g of aminophylline failed to shorten the prothrombin time of human subjects. Several other studies[5-8] with human subjects failed to demonstrate significant changes in prothrombin time after oral or intravenous administration of aminophylline. All studies reviewed above were done by using only one-stage methods for the determination of prothrombin. Either Quick's original technique or one of its numerous modifications were utilized.

Because of recent advances in the under-

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2 Scherf, D., and Schlachman, M., *Am. J. Med. Sc.*, 1946, **212**, 83.

3 Quick, A. J., *J. Biol. Chem.*, 1945, **161**, 33.

4 Holland, M. S., and Gross, E. G., *J. Iowa St. Med. Soc.*, 1948, **38**, 183.

5 Breytspraak, R. W., and Greenspan, F. S., *Am. J. Med. Sc.*, 1946, **212**, 476.

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standing of the blood clotting mechanism, and improved methods of analysis,[9-13] the problem was investigated further in this laboratory. It seemed especially important to take into account the newly recognized factor which accelerates the conversion of prothrombin to thrombin. This accelerator (Ac-globulin) is now recognized to be an integral part of the coagulation mechanism. A quantitative method for measuring its concentration has been developed.[12] It is essentially an adaptation of the two-stage method for prothrombin determination. The original two-stage method for prothrombin determination has also been modified[13] to account for variation in the activity of Ac-globulin. Using these newer techniques, we have, in this study, been able to demonstrate a definite effect of aminophylline on the coagulation mechanism in dogs.

Methods. Following a control period of 2 to 4 days, aminophylline was administered intravenously in varying dosages to healthy dogs maintained on a stock diet. Blood samples were drawn 4, 24, 48, and 72 hours after administration of the drug, and subsequently at 2 to 3 day intervals until normal values were obtained. To avoid thrombin formation we observed ordinary precautions in withdrawing blood and followed largely the technique described elsewhere.[14] One part of 3.2% sodium citrate was added to 9 parts blood. The plasma was removed following centrifugation and stored in a frozen state at —20°C until determinations were performed. Serial hematocrit determinations were made at first but because no significant variations were noted, this was later discontinued. The

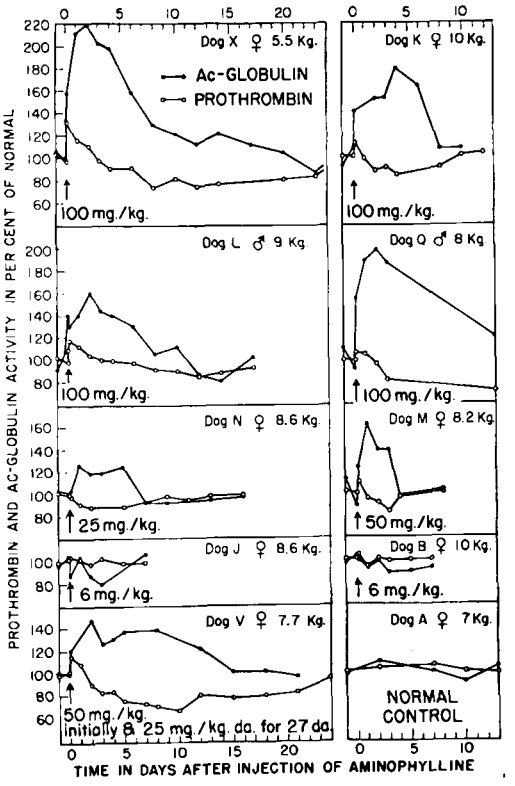

FIG. 1.
The protocol shows changes in plasma Ac-globulin and prothrombin concentration for 9 experimental animals and one typical control.

clotting time of whole blood, using the Lee-White method, was also followed at frequent intervals.

Prothrombin activity was determined by the modified two-stage method described by Seegers,[13] and Ac-globulin activity was estimated by the method of Ware and Seegers.[12] All determinations were compared to a standard control run simultaneously, so that minor corrections could be made for variations in reagents.

Results. Fig. 1 illustrates the results obtained with 8 dogs given single, intravenous doses of aminophylline, and with one dog given multiple intravenous injections. Prothrombin and Ac-globulin activities are expressed in per cent of normal control values. Expressed in units per cc of citrated plasma, normal prothrombin activity ranged from 170 to 185; Ac-globulin activity from 185 to 230.

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10 Owren, P. A., The Coagulation of Blood, Investigations on a New Clotting Factor, Oslo, 1947.

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

12 Ware, A. G., and Seegers, W. H., *J. Biol. Chem.*, 1948, **172**, 699.

13 Seegers, W. H., Blood Clotting and Allied Problems, Josiah Macy, Jr. Foundation, Feb., 1948.

14 Fahey, J., Olwin, J. H., and Ware, A. G., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 491.

All animals were first observed for a control period of a week or so to establish their particular normal (100%) levels.

Following the administration of 100 mg of aminophylline per kg body wt, a small but consistent rise in prothrombin activity was noted in 4 hours, falling to normal by 24 to 48 hours (dogs X, Q, L, and K). This was followed by a drop to approximately 15 to 30% below normal, gradually returning to normal in about 2 to 3 weeks. Ac-globulin activity rose sharply in 4 hours, reached a maximum in 48 to 96 hours, and then gradually fell to normal levels. Determinations on blood drawn 2 hours after administration of aminophylline to dog L showed that a significant response was present even at this time. This characteristic pattern was less evident with smaller doses (50 and 25 mg/kg body wt). Administration of 6 mg/kg body wt (approximate therapeutic dosage in man) was without effect on either prothrombin or Ac-globulin activity (dogs B and J).

Several dogs given salts of caffeine and theobromine intravenously in large single doses (100 mg/kg body wt), were found to respond in the same characteristic manner.

Dog V, given 50 mg of aminophylline per kg body wt initially, and then daily doses of 25 mg/kg body wt for 4 weeks, demonstrated that the elevation in prothrombin activity could not be maintained by repeated injections. Increased Ac-globulin activity persisted for a longer period but returned to normal in spite of continued administration of the drug.

As a matter of interest, plasma from several of these dogs was analyzed for prothrombin activity using the one-stage method. The technique originally described was carefully followed, using 100% plasma. Deviations from the normal values of 6 to 6.5 seconds were found with the large intravenous doses of the drug. This paralleled the results obtained using the two-stage method. For example, the prothrombin time of dog L fell to 5 seconds 4 hours after administration of aminophylline, remained at 5½ seconds for 96 hours, and increased to 7 seconds before returning to normal. On the other hand, dog J showed no deviation from normal.

No correlation could be found between the Lee-White clotting time of whole blood and variations in prothrombin and Ac-globulin activities. This has also been noted by other authors and is undoubtedly due to inadequacies of the method. A modified technique has been described by Blood and Patterson[8] which is probably a considerable improvement over the method we used. These investigators consistenly obtained a very narrow range of normal values for the clotting time of whole blood.

Summary. 1. Large doses of aminophylline, administered intravenously to dogs, produced a transient elevation in prothrombin activity, followed by a late hypoprothrombinemia with return to normal in about 2 to 3 weeks. Simultaneously, a more marked and persistent rise in Ac-globulin activity occurred. Small doses of 6 mg/kg body wt were without significant effect.

2. No correlation could be found between the Lee-White whole blood clotting time and variations in prothrombin and Ac-globulin activity.