most of the animals. This skin condition appeared to become progressively worse with time, and erythema was noticed around the scrotal area in the males, on the abdomen, and on the sides and under the jaws of animals of both sexes. Following the erythema, purpural skin hemorrhages were observed in the areas which had shown erythema. Extensive eschar formation followed the purpura. Usually the eschars were hard and dry, and the escharotic tissue was confined to small circumscribed areas. Exfoliation followed the escharotic stage, and there was a loss of hair around the areas previously occupied by the eschars. In some cases, ulceration followed exfoliation. When this occurred eschars were usually reformed, and exfoliation generally followed.

Those animals that were transferred to the control diet showed rapid improvement and recovery from the skin lesions. In cases where eschars were present, exfoliation and healing took place rapidly. Denuded areas began to show hair growth in 7 to 10 days, and these areas could not be detected at the close of the experimental period without close examination which would reveal numerous short hairs. Edema subsided within a week after the animals received the increased dietary magnesium, and the animals appeared in good condition by the close of the experiment.

Summary. Sexually mature rats maintained on a magnesium-deficient diet develop lesions of the skin characterized by erythema, purpural hemorrhages and eschars. The addition of magnesium to the diet results in recovery from the skin lesions. Other symptoms observed are essentially the same as in the immature animal deprived of magnesium. The increased length of time before the appearance of symptoms in older animals probably reflects increased body stores of magnesium with an increment in the time required to deplete the tissues of this element.

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Effect of Aminophyllin on the Coagulation of Human Blood.*

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Aminophyllin (theophyllin-ethylenediamine) is commonly used in the treatment of patients with heart disease, particularly when the coronary arteries are involved. Recently, emphasis has been put on the importance of intravascular coagulation of the blood on the course and prognosis following coronary occlusion. Observations have been published ascribing to aminophyllin a thromboplastic action. Because of the serious therapeutic implications of this finding, if confirmed, the present study was undertaken. Field, Larson, Spero, and Link[1] in reviewing the literature, found that a number of authors were of the opinion that the methylxanthines had an accelerating action on blood coagulation. They also reported that single oral doses of a number of methylxanthines produced hyperprothrombinemia and a decreased sensitivity to the action of 3,3′-methylenebis-4-hydroxycoumarin. In view of these findings they believed it possible that use of these drugs in man might produce a tendency toward thrombus formation. Link[2] again emphasized

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1 Field, J. B., Larsen, E. G., Spero, L., and Link, K. P., *J. Biol. Chem.*, 1944, **156**, 725.

2 Link, K. P., *Harvey Lectures*, 1943-44, **39**, 126.

these points and suggested that the use of the dilute (12.5%) plasma prothrombin time determination was preferred in attempting to evaluate any such changes in man. On the other hand, Poindexter and Meyers[3] found that oral administration of aminophyllin to a series of 10 patients had no effect on the plasma prothrombin time. Breytspraak and Greenspan,[4] in a small series of cases, found that oral and intravenous administration of aminophyllin produced no significant changes in the prothrombin time. Gilbert, Dey, and Trump[5] performed a series of experiments on both patients and animals and found no alteration in the clotting times, dilute and 100% plasma prothrombin times, or in the response to intravenous injection of heparin following the administration of xanthine drugs.

Material. A single intravenous injection of aminophyllin was given to 18 patients on the wards of the hospital. None had any liver disease or disorder of the blood-forming organs and in no case were drugs being taken which are known to affect the clotting of the blood. Following a control determination of clotting and prothrombin times, each subject was given 0.48 g of aminophyllin by intravenous injection over a 10-minute period. Clotting and prothrombin times were determined 30 minutes and one hour after completing the injection. Moderate tachycardia and diuresis were noted in most cases, but there were no other effects.

It is possible that aminophyllin has a slower action on blood clotting than would be detected following a single intravenous injection. Accordingly, a second group of 12 patients was studied to observe a more prolonged action of aminophyllin on blood coagulation. As in the first group, none of these patients had disease affecting the clotting of the blood nor were any taking drugs with known thromboplastic action.

Following an initial determination of clotting and prothrombin times, each patient was given 0.2 g of aminophyllin, by mouth, 4 times a day for 7 days. Two patients were given plain aminophyllin tablets, but because of nausea the remaining 10 patients took an enteric-coated preparation. At the end of the period of administration, a second determination of clotting and prothrombin times was done. With the enteric-coated preparations no ill effects were noted. Three patients, who had angina pectoris due to coronary insufficiency, obtained marked symptomatic relief while taking the aminophyllin.

Methods. These are described at length because strict attention to technical detail is essential to obtain accurate and consistent results. The clotting time was determined by a modification of the Lee-White method.[6] The equipment consisted of three 75 × 10 mm tubes in a water bath maintained at a constant temperature of 36 to 39°C. These tubes were cleaned chemically, rinsed in distilled water, and oven-dried before being used. Venous blood was obtained with as little trauma as possible and was collected in a dry syringe. The tourniquet was released before withdrawal of the blood. The stopwatch was started when one-half the required amount of blood had been withdrawn. One cc of this venous blood was placed into each of the 3 tubes. At 3 minutes the first tube was tilted and this procedure was repeated every 30 seconds until the tube could be tilted to the horizontal without having the blood flow down the side. At that time the second tube was similarly tilted and finally the third tube. The end point was considered to be that point at which the third tube could be tilted to the horizontal without any flow of blood.

The prothrombin time was determined by a slight alteration in the Link-Shapiro modification of Quick's method.[7] Five cc of venous blood was collected into a chemically clean test tube containing 0.01 g of solid potassium oxalate. After mixing, this sample was centrifuged and the plasma pipetted off and placed in a 75 × 10 mm test tube. Fifty

[3] Poindexter, C. A., and Meyers, L., *Quarterly Bull. Northwestern Univ. Med. School*, 1946, **20**, 130.

[4] Breytspraak, R. W., and Greenspan, F. S., *Am. J. Med. Sci.*, 1946, **212**, 476.

[5] Gilbert, N. C., Dey, F. L., and Trump, R. A., *J. Lab. and Clin. Med.*, 1947, **32**, 28.

[6] Lee, R. I., and White, P. D., *Am. J. Med. Sci.*, 1913, **145**, 495.

[7] Shapiro, S., *Exp. Med. and Surg.*, 1944, **2**, 103.

TABLE I. Clotting and Prothrombin Times Before and After Administration of 0.48 g of Aminophyllin by Intravenous Injection.

Diagnosis	Lee-White clotting time of blood (minutes)				Undiluted plasma prothrombin time (% of normal control)				12.5% plasma prothrombin time (% of normal control)			
	Initial	½ hr	1 hr	Final change	Initial	½ hr	1 hr	Final change	Initial	½ hr	1 hr	Final change
Acute rheumatic fever	7.5	8.5	7.0	— 0.5	100.0	123.5	110.5	+10.5	95.5	95.8	96.6	+ 1.1
Pneumonia	10.0	8.0	9.0	— 1.0	124.1	112.7	106.0	—18.0	84.1	92.3	89.6	+ 5.5
Secondary syphilis	6.5	7.5	7.5	+ 1.0	95.2	112.9	116.3	+21.1	100.2	98.2	107.5	+ 7.3
Diaphragmatic pleurisy	8.0	8.0	10.0	+ 2.0	116.4	107.6	108.7	— 7.7	81.1	79.1	80.1	— 1.0
Lymphadenitis	7.0	7.5	6.5	— 0.5	92.9	98.1	91.1	— 1.8	109.1	116.7	115.3	+ 6.2
Pneumonia	8.0	9.5	10.5	+ 2.5	107.6	100.5	105.9	— 1.7	93.1	98.0	85.1	— 8.0
Coronary insufficiency	9.0	8.5	9.5	+ 0.5	109.0	98.4	96.4	—12.6	104.1	93.4	90.6	— 3.5
Trichinosis	6.5	7.5	6.5	± 0.0	104.0	98.2	101.3	— 2.7	103.9	101.9	104.8	+ 0.9
Pneumonia	6.5	7.5	6.5	± 0.0	105.6	101.2	104.3	— 1.3	98.7	101.4	102.1	+ 3.4
Lymphoblastoma	5.5	5.5	5.0	— 0.5	86.1	91.3	95.1	+ 9.0	91.0	97.3	99.2	+ 1.2
Pneumonia	6.5	6.5	7.0	+ 0.5	105.1	111.8	112.7	+ 7.6	97.3	104.9	106.4	+ 9.1
Psychoneurosis	5.0	4.5	4.5	— 0.5	92.3	96.1	91.7	— 0.6	87.7	92.4	93.9	+ 6.2
Pneumonia	7.0	7.0	7.5	+ 0.5	114.8	114.8	124.2	+ 9.4	111.5	100.7	101.4	—10.1
Coronary heart disease	7.0	6.5	6.0	— 1.0	98.8	96.1	106.6	+ 7.6	93.3	100.8	98.6	+ 5.3
Pneumonia	9.5	9.5	10.0	+ 0.5	94.2	95.2	104.7	+10.5	87.4	85.7	85.2	— 2.2
Nasal polyposis	9.0	9.5	9.0	± 0.0	99.4	96.0	102.8	+ 3.4	98.8	100.8	89.7	— 9.1
Amebic dysentery	8.5	9.0	8.5	± 0.0	92.7	98.6	89.0	— 3.7	111.6	105.3	104.2	— 7.4
Duodenal ulcer	7.0	7.5	8.0	+ 1.0	93.6	96.4	97.1	+ 3.5	94.7	88.8	90.0	— 4.7

TABLE II.
Clotting and Prothrombin Times Before and After Administration, by Mouth, of 0.2 g of Aminophyllin, Four Times Daily for One Week.

Diagnosis	Lee-White clotting time of blood (min)			Undiluted plasma prothrombin time (% of normal control)			12.5% plasma prothrombin time (% of normal control)		
	Initial	Final	Change	Initial	Final	Change	Initial	Final	Change
Coronary heart disease	7.5	7.5	± 0.0	102.7	87.0	—15.7	106.2	105.1	— 1.1
,, ,, ,,	5.5	9.5	+ 4.0	97.2	111.2	+14.4	94.1	99.4	+ 5.3
,, ,, ,,	5.0	5.5	+ 0.5	107.1	107.2	+ 0.1	91.4	94.2	+ 2.8
,, ,, ,,	8.5	8.5	± 0.0	109.8	98.2	—11.6	103.4	96.3	— 7.1
Hypertensive vascular disease	6.0	4.5	— 1.5	91.0	84.3	— 6.7	96.7	91.8	— 4.9
Cor pulmonale; pulmonary fibrosis	7.0	8.0	+ 1.0	78.7	97.1	+18.4	84.5	88.7	+ 4.2
Tuberculous pericarditis	5.5	5.5	± 0.0	75.4	100.5	+25.1	91.4	96.2	+ 4.8
Coronary heart disease	4.5	8.0	+ 3.5	88.4	87.5	— 0.9	88.4	90.0	+ 1.6
Thoracic neoplasm	6.0	8.0	+ 2.0	87.9	96.7	+ 8.8	93.1	99.3	+ 6.2
Hypertensive cardio-vascular disease	6.0	8.0	+ 2.0	113.6	109.8	— 3.8	107.0	118.3	+11.3
Regional enteritis	5.5	7.0	+ 1.5	98.9	113.1	+14.2	116.7	101.7	—15.0
Coronary heart disease	4.5	6.5	+ 2.0	101.1	105.8	+ 4.7	84.4	96.0	+11.6

mg of desiccated rabbit lung† was placed into 2.5 cc of normal saline solution and stirred for 10 minutes in a water bath at 56 to 58°C. The mixture was then cooled at 26°C for 1 minute. Two and one-half cc of 0.025 molar calcium chloride were added to this thromboplastin suspension and the resulting mixture stirred for an additional 4 minutes. The thromboplastin-calcium chloride mixture was then centrifuged for 5 minutes, the supernatant decanted and placed in 0.2 cc amounts into 75 × 10 mm test tubes.

The actual test was performed by adding 0.1 cc of the plasma to the 0.2 cc of thromboplastin-calcium chloride mixture and stirring the solution vigorously with a wire loop. The stop-watch was started as the first drop of plasma entered the thromboplastin tube. The end point was that time at which a firm clot formed across the wire loop. An identical test was also done using 12.5% plasma. This was obtained by diluting a sample of the plasma 1 to 8 with normal saline solution.

The strength of the thromboplastin was found to vary considerably with different lots of the preparation and for this reason a control plasma prothrombin time was determined on a normal subject at the time each patient was tested. In order to orient the results in relation to a common baseline, the prothrombin times are all expressed as the percentage of the normal control for a given day. Duplicate determinations were made on both undiluted and 12.5% plasma, in the control as well as in the subject whose blood was being studied.

Results. In the group given a single dose of 0.48 g of aminophyllin by intravenous injection there was no significant change in the clotting times. The individual and average variations were slight. The average initial clotting time was 7.5 minutes. After one-half hour this had increased to 7.7 minutes; the final average was the same, indicating an increase in the clotting time of 0.2 minute (Table I). In the patients on prolonged oral aminophyllin intake there was also very little individual or average variation. The average initial clotting time was 6.0 minutes and the final 7.2 minutes, indicating an increase of 1.2 minutes (Table II).

In the patients who had received aminophyllin by intravenous injection there was little difference in the prothrombin times before and after administration of the drug. The average initial undiluted plasma prothrombin time was 101.8%, at one-half hour it was 102.8%, with a final average of 103.5%, indicating an overall increase of 1.7%. Similar results were obtained using 12.5% plasma. In this case the initial average was 96.8%, with a one-half hour average of 97.4% and a final average of 97.2%. This was a final increase of 0.4%. In the group of patients receiving aminophyllin orally for one week there was again little variation in the prothrombin times before and after drug administration. The initial undiluted plasma determination averaged 96.0% with a final average of 99.9%, indicating an increase of 3.9%. Similarly, in the determination on 12.5% plasma the initial average was 96.4% and the final 98.1%; this was an increase of 1.7%. In none of the results were the differences statistically significant.

Summary. Aminophyllin was given in a single intravenous injection to a group of 18 patients, and orally 4 times daily for a week to a second group of 12 subjects.

There were no statistically significant changes in the clotting time of the blood or in the undiluted or 12.5% (diluted) plasma prothrombin times following the administration of aminophyllin.

† Prepared by the Maltine Company, New York.