in mice and the studies of Harrow, *et al.*,[3] on the mechanism of detoxification of orally administered *p*-aminobenzoic acid by the formation of the acetylated derivative. However, as a further control in the present experiments, some of the animals were given a single subcutaneous dose of 25 mg of *p*-aminobenzoic acid 30 minutes prior to the injection of sulfapyridine.

The toxic effects of the 25 mg dose of sodium sulfapyridine on normal animals and animals receiving *p*-aminobenzoic acid were compared, and the results of this type of experiment are illustrated in Table II. It will be seen that *p*-aminobenzoic acid exerts no appreciable effect on the acute toxicity of sulfapyridine when administered in this manner. The time of onset and the duration of convulsions in the group receiving *p*-aminobenzoic acid did not differ significantly from that in the control group. The single animal that survived the 25 mg dose of sulfapyridine did so only after several hours of severe convulsions.

Summary. 1. *P*-aminobenzoic acid (given subcutaneously) was found capable of nullifying the curative effect of sulfapyridine (given *per os*) for type I pneumococcus infection in mice. 2. *P*-aminobenzoic acid had no observable effect upon the immediate fatal toxicity of sulfapyridine for mice.

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Effect of Intramuscular Injection of Sodium Citrate on the Prothrombin Time of the Blood.*†

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Neuhof and Hirshfeld[1] observed that intramuscular injections of sodium citrate caused an increase in the coagulability of the blood. A fall in the coagulation time occurred within 40 minutes of an intragluteal injection of 30 cc of 30% citrate and was maintained

[3] Harrow, Benj., Power, F. W., and Sherwin, C. P., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 422.

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† The authors gratefully acknowledge the kind advice of Professor Arthur Wright.

[1] Neuhof, H., and Hirshfeld, L., *Ann. Surg.*, 1922, **76**, 1.

at a lowered level for 1 hour. It returned to its initial value in 24 hours. Hemoglobin estimations, red blood cell counts, carbon dioxide-combining power, oxygen and calcium analyses showed no change after the injection of sodium citrate. The authors accounted for the hypercoagulability on the basis that citrate destroyed the blood platelets from which was liberated a blood-coagulating substance.

De Souza and Hocking[2] investigated the work of Neuhof and Hirshfeld. Their studies, however, followed along the lines of Mills,[3] who had previously demonstrated a direct relationship between alkali reserve and blood coagulation. Thus it was shown that sodium citrate given intramuscularly or intravenously lowered the coagulation time of blood, and that the change was accompanied by an increase in the alkali reserve. Intraperitoneal injections of the salt gave the same effect more slowly, probably due to slower absorption. This excluded the possibility of local muscular damage with release of thromboplastin into the blood being the causative factor in the increased coagulability.

These authors also showed that the alkali reserve was increased by the oxidation of sodium citrate to sodium bicarbonate. Equivalent intramuscular injection of sodium bicarbonate raised the alkali reserve, but the effect on coagulation was more transient. Conversely, ammonium chloride lowered the alkali reserve and raised the coagulation time of the blood. In a series of exclusive experiments it was also shown that hypertonicity of the solution, or the volume injected did not in themselves effect the coagulability of the blood.

Purpose. Due to recent improvements in the method for the estimation of prothrombin it was decided to extend these blood coagulation studies to determine the effect of intramuscular injections of sodium citrate, sodium chloride and potassium chloride on the circulating prothrombin.

Methods. Fourteen normal dogs received, in various concentrations and in different volumes, intramuscular injections of one of the substances mentioned above. Prior to the injection a blood sample was taken on which the following determinations were made: (1) prothrombin time, (2) tube coagulation time, (3) calcium coagulation time, (4) capillary coagulation time, and (5) hematocrit. Following the injection, blood samples were taken every 30 minutes for a period of 2 hours and the estimations repeated. Blood was drawn by needle puncture through the skin from the unanesthetized

[2] de Souza, D., and Hocking, F. D. M., *J. Physiol.*, 1934, **83**, 49.

[3] Mills, C. A., *J. Biol. Chem.*, 1921, **46**, 197.

animal. In addition, 2 control animals which received no injections were subjected to the same tests at the same time intervals.

The prothrombin time determinations were done by the method of Quick. Only freshly prepared thromboplastin was used. The tube coagulation test was performed in a small tube (method of Lee and White). The calcium coagulation time was carried out in the same manner as the Lee and White method except that 3 drops of 1% calcium chloride were previously placed in the tube. The capillary coagulation tests were done in capillaries of uniform bore. Hematocrit determinations were done in the graduated tubes provided for that purpose. During the test period the animals received no water, food or medication.

Results. Six animals whose weight varied from 9 to 17 kg received intramuscular injections of sodium citrate. The amount injected varied from 1.50 g to 8.50 g and averaged from 0.125-0.50 g per kilo. The volume of solution containing this salt was about 50 cc. All solutions were therefore hypertonic.

The initial average prothrombin time was 11.8 seconds. Thirty minutes after the injection the average fall in prothrombin time was 17%. The prothrombin time continued to fall so that at the expiration of 90 minutes the average prothrombin time was reduced 24%. In dog 3, at the end of one hour, the prothrombin time was reduced 50%. The coagulation time as determined by the method of Lee and White was lowered within 60 minutes to 48% of its original value. The calcium coagulation time was carried out in each sample to make certain that the injection of sodium citrate had not materially lowered the calcium content of the blood. Since the results of this test did not vary from the Lee and White method, they

TABLE I.
Effect on Blood of Intramuscular Injection of Various Salts.

Dog	Wt in kg	Injection per kg	Prothrombin time (sec.) 0	½	1	1½	2 hr	Coagulation time (min.) Lee and White method 0	½	1	1½	2 hr
1	12.0	Na citrate 0.125g	12.2	9.2	11.2	9.4	9.0	3:25	1:50	1:25	2:05	1:50
2	9.0	" " "	13.0	12.0	12.0	10.0	10.0	4:00	2:25	2:10	2:10	3:00
3	9.0	" " 0.33	12.5	8.0	6.5	9.7	11.0	2:30	1:10	0:30	0:40	1:15
4	17.0	" " 0.50	11.0	11.0	9.0	9.0	10.0	3:20	1:40	1:20	1:40	1:40
5	12.5	" " 0.50	12.0	11.0	9.0	9.0	11.0	3:00	1:30	0:30	1:50	1:50
6	17.0	" " 0.50	10.0	6.6	7.3	7.0	7.0	3:00	1:10	1:40	1:20	1:20
		Avg effect	11.8	9.6	9.1	9.0	9.6	3:12	1:37	1:32	2:02	2:09
7	9.0	Normal dog	9.0	9.0	9.2	9.4	9.5	1:30	1:00	1:40	2:30	2:00
8	14.0	" "	15.5	16.0	15.5	15.5	15.7	2:00	2:40	2:40	2:00	2:00
9	10.5	NaCl 0.64	8.0	8.0	8.0	10.0	8.0	2:30	2:10	2:10	1:30	1:40
10	12.5	" 0.50	11.1	11.9	13.0	12.0	14.2	1:15	1:15	1:15	1:05	0:40
11	11.0	KCl 0.50	8.5	9.2	9.2	9.8	9.2	1:10	2:20	1:50	2:15	1:30

are not shown in Table I. Similarly, the data on the capillary coagulation time are not included in the table, since the results paralleled those of the tube coagulation time. The hematocrit estimations showed no characteristic variations and the results, accordingly, are not given in the table.

In 2 normal dogs used as controls, 5 prothrombin estimations over a 2½-hour period showed no greater variation than 0.5 seconds in the prothrombin time. In addition, 3 other control animals were used; 2 animals were injected with sodium chloride and 1 animal with potassium chloride. None of these animals developed the characteristic fall in the prothrombin and coagulation times, that were invariably obtained when sodium citrate was injected. The hematocrit determinations in the normal controls, as well as in the animals injected with sodium or potassium chloride, showed no characteristic variations.

Summary. Intramuscular injections of sodium citrate caused a lowering of the clotting time of blood as determined by the capillary and Lee and White methods. The increased coagulability was accompanied by a fall in the prothrombin time in the blood, as determined by Quick's method. Control animals, and those injected with sodium or potassium chloride, did not show a lowering of the prothrombin time.

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An Error in Measuring Changes in Plasma Volume After Exercise.

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The plasma volume under basal conditions may be satisfactorily determined by the dye method. The original dye method described by Keith, Rowntree, and Geraghty,[1] has been rendered more accurate by the use of better methods of colorimetry and by the calculation of the volume from the disappearance slope of the dye. It is impossible to measure rapid changes in plasma volume which last only a short

[1] Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, **16**, 547.