in 1931–1932 had been inbred for some time before they were received in this laboratory in 1928. From then until 1935 they were mated in a random fashion by selecting healthy stock without regard to relationship.

In spite of the failure to significantly increase uniformity in our colony by this means, it has been definitely shown by others that considerable improvement in accuracy results from the use of a carefully bred and tended stock of animals (5, 11).

The slopes of the regression lines for sulpharsphenamine, arsphenamine and mapharsen have been computed for a few assays using male rats in the majority of tests, and are given here as a matter of interest. The average values of b were as follows; for sulpharsphenamine 9 $\pm$2.1, for arsphenamine 10 $\pm$1.3 and for mapharsen 9 $\pm$0.97.

SUMMARY

1. Two hundred tests for the toxicity, and seventy-six tests for the trypanocidal activity of commercial samples of neoarsphenamine are reported. Toxicity and trypanocidal activity are expressed in terms of the International Standard Neoarsphenamine.

2. Differences are shown to exist in the toxicity and activity of the products of different manufacturers.

3. The least toxic neoarsphenamines frequently have the highest trypanocidal activity, as judged by these assays.

4. Some brands are more variable from batch to batch in their toxicity than others.

5. The toxicity of four lots of Canadian Standard Neoarsphenamine, and the trypanocidal activity of two lots are reported.

6. A one-dose method of assay, using curve numbers in calculating results, may be employed with the rats used in this laboratory.

7. The effect of inbreeding on the slope of the dosage-response curve is demonstrated.

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Intravenous Toxicity of Heparin-Sodium Sulfapyridine Combinations and Protective Action of Barbiturates*

By N. A. David, N. M. Phatak, H. Donnell and H. Vehrs†

INTRODUCTION

Murray and Best (1) first demonstrated the usefulness of heparin in the prevention of post-operative thrombosis. Since then studies on the use of heparin in subacute bacterial endocarditis have also been reported (2, 3). In this condition it would seem desirable to give one of the newer chemotherapeutic agents simultaneously with the intravenous administration of heparin in order that the blood stream may be cleared of the invading micro-organisms and preventing at the same time further deposition of thrombotic masses on the heart valves. However, no experimental information has thus far been reported indicating the safety of such a procedure.

EXPERIMENTAL

Prior to the clinical use of heparin and sodium sulfapyridine mixtures intravenously in cases of

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subacute bacterial endocarditis, we have studied the safety of such a procedure in seven experimental dogs. These animals were given intravenously 5 per cent solution of sodium sulfapyridine in either physiological saline or in molar sodium lactate (Hartmann's solution), and mixed with 2 cc. heparin (2000 units) per 100 cc. of the mixture. Since the usual recommended therapeutic dose of sodium sulfapyridine for man is 60 mg. per Kg. it would seem that 10 times this amount should show definite toxic manifestations in experimental animals; therefore, we injected our dogs with 0.6 Gm. per Kg. of sodium sulfapyridine plus 2 cc. of heparin added per 100 cc. of the mixture. Solutions were warmed to body temperature and injected by gravity at the rate of 2–3 cc. per minute. The results of these experiments showing the levels of the blood sulfapyridine and the plasma-CO_2 values for several hours after injection and the response shown by the animal are summarized for 6 of the 7 dogs in Table I. No blood chemistry studies were made on the first dog treated; observations on this animal are reported in the protocol below.

PROTOCOL

February 8, 1940 Dog No. 1, Spotty Male, 9.1 Kg.

11:40 A.M. Solution made under aseptic conditions as follows:

Heparin Solution (1000 units per cc.)	4.00
Sodium sulfapyridine	10.00
Physiological Saline *q. s. ad*	200.00

8:00 P.M. Solution inspected, appeared clear; warmed to 37 degrees C.

8:15 P.M. Intravenous injection commenced and continued at rate of 2–3 cc. per minute by gravity drip.

8:45 P.M. Salivation and occasional retching noted.

9:00 P.M Injection of 110.0 cc. of the 5% sodium sulfapyridine-heparin in saline solution completed. Total amount sodium sulfapyridine given, 5.50 Gm. (0.6 Gm. per Kg.)

9:02 P.M. Vomited, defecated and urinated.

9:10 P.M. Sudden onset of severe clonic convulsion with some opisthotonus characterized by extension of limbs with jerking movements and constant snapping of jaws; lasted for 4 minutes until ether applied.

9:14 P.M. Under light ether anesthesia, spasmodic convulsions continue with less severity, jerking movements of limbs at rate of 40 to 51 per minute.

9:18 P.M. Dog completely anesthetized, convulsions cease, ether removed.

9:27 P.M. Second convulsion began suddenly; ether applied at once to point of anesthetization and continued until 9:40 P.M. Convulsion disappeared under anesthesia.

9:40 P.M. Rectal temperature, 39.2 degrees C.

9:45 P.M. Third convulsion began, less intense than previous; ether anesthesia started 1 minute after convulsion began and light anesthesia continued three minutes until convulsion ceased.

10:00 P.M. Fourth convulsion, less intense; light ether anesthesia given for several minutes. Dog relaxed and continued to sleep after anesthesia removed.

11:00 P.M. Dog has continued sleeping, completely relaxed. No further convulsions appeared up to 12:30 P.M. when observations were concluded.

February 9, 1940

8:00 A.M. Dog appears well, drinks warm milk and water. Returned to animal quarters. Survived.

The most constant side effects noticed after the oral or intravenous administration of toxic doses of sodium sulfapyridine are nausea, vomiting, generalized clonic and tonic convulsions, acidosis, drug fever, coma and death. Moderate or even therapeutic amounts cause nausea, salivation, vomiting and often mild jerky muscular tremors. Since we administered the mixtures intravenously, the toxic effects we noted must be attributed to central nervous system stimulation rather than to local gastric irritation or to a reflex therefrom. In all animals either repeated administrations of ether inhalation or a single parenteral injection of 15 to 20 mg. per Kg. of pentobarbital was sufficient to prevent the convulsive seizures and to spare the muscular exhaustion of the animals. However, ether administration alone failed to completely protect the animals. Intraperitoneal injection of the above amounts of sodium pentobarbital given 20 to 30 minutes previous to or soon after completion of the injection of sodium sulfapyridine not only minimized the toxicity of the drug but completely protected the animals from convulsive seizures and certain death. When sodium pentobarbital was given in conjunction with intravenous administration of sodium sulfapyridine-heparin mixtures, the dogs invariably showed a complete recovery to normal in less than 24 hours. This use of the barbiturate (a "short-acting" one) is not only spectacular but a definite life saving measure and should be of therapeutic value in counteracting the toxic effects of sulfanilamide derivatives when given to man.[1]

[1] Adriani (4) has recently reported that the use of sub-anesthetic doses of several barbiturates in rats receiving sulfanilamide serves to increase the toxicity of the latter drug. It is to be noted that we employed only hypnotic doses of the barbiturate and used sulfapyridine rather than sulfanilamide.

Table I.—Response of Dogs and Changes in the Blood Sulfapyridine and Plasma CO_2 Levels Following Intravenous Injections of Sodium Sulfapyridine-Heparin Mixtures

Dog No.		Fasting	Time ½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	8 hrs.	Drugs Given and Response of Dogs
2	Sulfapyrid.		36.0	42.8	48.1	43.5	43.5		38.8		0.6 Gm./Kg. 5% Sod. Sulfapyr. in saline.
	Plasma CO_2	33.6	27.9	38.0	50.4	53.7	57.3		51.5		No Heparin. 25 mg./Kg. Pentobarbital given I.P. 1½ hrs. previously. No reaction. Died 48 hrs. later of pneumonia.
3	Sulfapyrid.		53.0	50.0	49.2						0.6 Gm./Kg. 5% Sod. Sulfapyr. in saline with Heparin. Ether only to prevent convulsions. Died 2 hrs. after start of injection; excessive rise of temperature.
	Plasma CO_2	60.5	58.5	68.3	66.2						
4	Sulfapyrid.		46.3	47.7	46.3	47.0	44.9				0.6 Gm./Kg. 5% Sod. Sulfapyr. in glucose-saline with Heparin. Mild convulsions controlled by ether but necessary to give 20 mg./Kg. Pentobarbital after 2 hrs. O.K. Survived.
	Plasma CO_2	60.5	48.2	45.0	68.3	54.4	55.3				
5	Sulfapyrid.		40.0	41.4	40.7	37.0	33.9		28.6		0.6 Gm./Kg. 5% Sod. Sulfapyr. in molar sod. lactate with Heparin followed in ½ hr. by 14 mg./Kg. Pentobarbital I.P. O.K. Survived.
	Plasma CO_2	50.2	68.8	81.1	70.8	64.8	65.9		58.5		
6	Sulfapyrid.		39.6	33.9	33.1	28.3	27.2	22.4			0.6 Gm./Kg. 5% Sod. Sulfapyr. in molar sod. lactate with Heparin followed in ½ hr. by 14 mg./Kg. Pentobarbital I.P. O.K. Survived.
	Plasma CO_2	60.0	70.8	62.7	57.6	69.9	60.0	56.4			
7	Sulfapyrid.		38.5	40.9	32.5	31.8	32.5	24.9	23.8	21.4	0.6 Gm./Kg. 5% Sod. Sulfapyr. in molar sod. lactate with Heparin. 15 mg./Kg. Pentobarbital I.P. ½ hr. before injection started. O.K. Survived.
	Plasma CO_2	58.5	75.9	60.5	55.6	59.6	65.9	67.9	62.7		

Use of molar sodium lactate with heparin-sodium sulfapyridine mixtures materially alleviates acidosis and serves as an efficient buffer as evidenced by plasma CO_2-capacity determinations. In dogs the use of molar sodium lactate maintains a plasma CO_2 level between 60 to 80 volumes per cent, which, in its absence, remains at 40 to 50 volumes per cent. It alone, however, does not prevent the appearance of toxic effects of sodium sulfapyridine but lessens the severity and mitigates the after effects.

A mixture of sodium sulfapyridine and heparin remains stable in solution for at least 6 to 10 hours and often up to as long as a week depending on the condition of storage. Heparin and sodium sulfapyridine mixtures are not stable in either glucose or glucose-saline vehicle.

CONCLUSIONS

From our studies we can say that heparin-sodium sulfapyridine mixtures may be safely administered intravenously as a continuous drip.

Use of sodium pentobarbital, orally or parenterally in hypnotic doses, either previous to treatment or simultaneously with it, is not only beneficial but advisable as an adjunct to reduce the untoward manifestations of sodium sulfapyridine therapy.

Use of molar sodium lactate is recommended in preference to either physiological saline or glucose as a vehicle for the parenteral administration of heparin-sodium sulfapyridine to counteract the acidosis.

We wish to thank Dr. K. K. Chen, Lilly Research Laboratories, Indianapolis, Indiana, for sending us generous supplies of sodium sulfapyridine and sodium pentobarbital. The heparin we used was obtained in 10 cc. vials (1000 units per cc.) from the Connaught Laboratories, Toronto, Canada.

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Fritz Pregl (1869–1930) was awarded the Nobel Prize for Chemistry in 1923 for his work on the micro-analysis of organic substances, just ten years after he explained the fundamentals of his methods at a meeting of naturalists and physicians in Vienna.