activity remaining in 10 minutes, showed a marked increase with addition of hyaluronidase to 45.6% activity remaining in 10 minutes. The preliminary study suggests definite advantages to the subcutaneous route over the intramuscular particularly for clinical study of effective blood flow because of the ease of injection, deviation of subsequent clearance rates and the inherent liability of unabsorbed radiation in deep tissue. It is possible that the increased rate of clearance subcutaneously with hyaluronidase represents marked diffusion rather than absorption.

The results of arterial and venous clamping of the common iliac vessels supports the recent view of Cullen *et al.*(7) that there is no benefit in concomitant venous ligation in acute arterial occlusion as was suggested by the early work of Makius(8) and Brooks *et al.*(9).

Summary. A study of the effect of physical factors on radiosodium clearance in dogs is reported. The average clearance rate in 25 control intramuscular injections was 59.4% activity remaining after 10 minutes. Decreases in intramuscular clearance rates were demonstrated with application of ice-salt mixtures, radiant heat and pressure cuffs. The average subcutaneous clearance rate in 10 control studies was 75.8% activity remaining in 10 minutes. This rate of clearance was increased with the addition of hyaluronidase. The clearance of radiosodium from a part does not appear as a straight line function when plotted and the calculation of per cent activity remaining per unit time is suggested as an expression for determination of clearance rates. The present study entirely supports the view of Kety that this technic is applicable to measure the effective circulation of a tissue and indicates the need for further study to quantitate the procedure for clinical application.

7. Cullen, M. L., Streppacher, L. G., Greenspan, B., Milliken, H. E., Moore, G. W., and Morris, G. C., *Surg., Gynec. and Obst.*, 1949, v89, 722.

8. Makius, G. H., Bradshaw Lecture, London, 1914.

9. Brooks, B., Johnson, G. S., and Kirtley, J. A., Jr., *Surg., Gynec. and Obst.*, 1934, v59, 496.

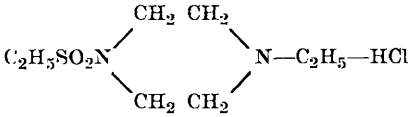
Received May 2, 1950. P.S.E.B.M., 1950, v74.

Activity of Ethyl-l-Ethanesulfonyl-4-Piperazine Hydrochloride on Traumatic Hemorrhagic Shock on the Dog. (17926)

D. BOVET* AND J. FOURNEL (Introduced by A. J. Goldforb)

From the Pharmaceutical Research Division, Rhône Poulenc, Paris.

Researches, in recent years, on traumatic shock therapy, have led us to examine the effects of the following substances on shock induced in the rat by means of the drum technic (Noble and Collip), with polyvinylpyrrolidone(1), some simple aliphatic amides (2), and the protective effect exercised by a new synthetic compound, ethyl-1-ethanesulfonyl-4-piperazine (3885 R.P.)(3).



In previous experiments we tested the effect of ethyl-1-ethanesulfonyl-4-piperazine (3885 R.P.) on shock produced in the mouse, rat and guinea pig, then decided to carry out further experiments on the dog. We used the technic of Wiggers, Ingraham and Dilles (4) which produced an irreversible shock following a prolonged hemorrhagic hypotension period. The animals were treated as

* Address: Instituto Superiore di Sanita—Roma, (Italy).

1. Bovet, D., Couvoisier, S., and Ducrot, R., C.R., 1947, v224, 70

2. Bovet, D., Couvoisier, S., Ducrot, R., and Jacob, R., C.R., 1947, v224, 496.

3. Bovet, D., Couvoisier, S., Ducrot, R., and Jacob, R., C.R., 1947, 227, 1423.

described by Wiggers, under morphine or chloralose anesthesia. By extensive bleeding the blood pressure was lowered to 40 mm of kg maintained for a given time by a series of successive hemorrhages and reinjections. A total reinjection of the removed blood immediately followed the period of hypotension. The wounds were treated locally with sulfanilamide and the surviving animals were given penicillin. To minimize the seasonal or other variations which constitute the principal obstacles in this type of research, the experiments were repeated in the spring and in the autumn, and parallel experiments have been carried out in Paris and in Rome. One hundred dogs were used. The duration of hypotension was prolonged for more than the 90 minutes reported by Wiggers(4), and there resulted a larger proportion of irreversible shock in the control animals.

In the first series of experiments (I, II, III), 16 control dogs were subjected to 2 hours hypotension, with a mortality of 94%. In series IV, the control dogs exhibited a higher resistance and the period of hypotension was extended to 2½ hours.

Of the different compounds tested, polyvinylpyrrolidone and tuamine (2-amino-heptane hydrochloride), did not give any protection against severe hemorrhagic shock. Two synthetic amides, the N-N-bis-diethylamino-ethyl-ethanesulfonamide hydrochloride (3720 R.P.) and ethyl-1-ethanesulfonyl-4-piperazine hydrochloride (3885 R.P.) gave protection and the results are given below.

Compound 3885 R.P. was administered to the experimental dogs at different times, either before or after the first major hemorrhage. The most favourable results were obtained when the injection of the product immediately followed the hemorrhage. The results were less constant, probably due to rapid elimination when the compound was injected before hemorrhage, for its effectiveness decreased rapidly during the hypotensive phase.

The results are shown in Table I. There was an 88% mortality among the control animals; and 15% among the animals treated before or immediately after the hemorrhage. The period of survival was much longer in the protected animals than in control animals; 60% of the latter died within 6 hours, whereas not a single one of the treated animals died in this period.

Death from shock never occurred more than 24 hours after the hemorrhage; there was only one case of a later death in a treated animal, and this appeared to be the result of an embolism.

The untreated animals presented a clinical picture corresponding exactly with that described by Wiggers and Ingraham(3,4). Irreversible shock was constantly preceded by a marked fall of blood pressure at the end of hypotension, and by emission of bloody faeces. The treated animals practically never presented these characteristic symptoms of shock.

Discussion. On traumatic shock in the dog, we demonstrate the protective activity of certain aliphatic sulfonamides. This phenomenon was previously observed by one of us in experiments conducted with mice, rats and guinea pigs(3).

Hemorrhagic shock produced in the dog by the method of Wiggers, Ingraham and Dille, was interesting for 3 reasons: 1. The analogy between the symptoms of this type of experimental shock and the clinical shock is well established. 2. The conditions utilized in these experiments were extremely severe; with a mortality of 80 to 100% of control animals. 3. All previous attempts for protection against hemorrhagic shock have resulted in incomplete and limited protection.

Using ethyl-1-ethanesulfamide-4-piperazine hydrochloride (3885 R.P.) either before or immediately after the hemorrhage, it was possible to prevent or to retard the symptoms of shock such as cardiovascular collapse, prostration and intestinal hemorrhage, and to assure a higher percentage of survivals.

Concerning the mode of action of 3885 R.P., it has been previously reported that this compound cannot be classified in any known group of pharmacodynamic agents; it

4. Wiggers, H. C., Ingraham, R. C., and Dille, J., *Am. J. Physiol.*, 1945, v143, 126; Ingraham, R. C., and Wiggers, H. C., *Fed. Proc.*, 1945, v4, 36; Wiggers, H. C., and Ingraham, R. C., *J. Clin. Invest.*, 1946, v25, 30; Wiggers, H. C., and Ingraham, R. C., *Am. J. Physiol.*, 1946, v146, 431.

TABLE I.
Activity of Ethyl-1-Ethanesulfonyl-4-Piperazine Hydrochloride on Traumatic Hemorrhagic Shock in the Dog.

Series		Duration of hypotension, hr	Narcosis*	Controls			Experimental			
				No. of animals	Survival after re-injection, hr†	Mortality, %	No. of animals	Treatment	Survival after re-injection, hr	Mortality, %
I	Rome May, 48	2	M	5	0, 0, 3, 5½	100	5	15 min. before hemor.	12, 4 S	20
II	Paris Oct., '48 to Jan., '49	2	C	6	1-1/6 1-2/6, 2-1/2, 2-5/6, 1 S	83	6	1 min. after hemor.	10, 5 S	16
							5	20 min. after hemor.	9, 23½, 3 S	40
III	Paris May, '49	2	C	5	2, 2-1/2, 3, 5, nearly 18	100	5	20-60 min. before hemor.	20, 4 S	20
IV	Rome Oct., '49	2½ ‡	C	10	1/12, 1/8, 1/6, 1/6, 1/6, 3/4, 12, 12, 2 S	80	10	1 min. after hemor.	12, 7 S	10

* M = Morphine (5 mg/kg s.c.). C = Chloralose (100 mg/kg i.v.).
† S = Number of survivals.
‡ 5 control dogs, subjected for the same period and hypotensive-hemorrhagic shock of 2 hours, had a mortality of only 20%.

does not seem possible to explain the protective effect in shocked dog by any action in the cardiovascular system in normal animals.

The experiments do not exclude the possibility of a pharmacodynamic antagonism for one of the hypothetical toxic substances responsible for shock.

Received May 9, 1950. P.S.E.B.M., 1950, v74.

Prothrombin Utilization during Clotting: Comparison of Results with the Two-Stage and One-Stage Methods.* (17927)

ROBERT D. LANGDELL, JOHN B. GRAHAM,† AND K. M. BRINKHOUS
From the Department of Pathology, University of North Carolina, Chapel Hill.

It has been recognized for decades that prothrombin disappears from blood during clotting, but that traces of this clotting factor may persist in serum for days(1). With the development several years ago of the 2-stage method for determining prothrombin in fibrinogen-free systems(2), further studies of prothrombin and the dynamics of clotting became possible. One of us, using this method, determined the rate of prothrombin consumption during clotting of normal, hemophilic and platelet-poor bloods(3,4). Originally, the one-stage test could be used only for the study of plasma, since no separate source of fibrinogen was provided. Recently this procedure has been modified so that it can be applied to serum(5). With the modified one-stage test, serums from normal, hemophilic and platelet-poor bloods have been investigated by several workers. While the results seemed to confirm in a general way the findings with the 2-stage test, certain discrepancies were apparent.

The present study was undertaken to compare prothrombin utilization during clotting, as indicated by the 2 methods, and to determine, if possible, the basis for the discrepancies observed. Simultaneous one-stage and 2-stage determinations of prothrombin activity were performed on a series of bloods during and after clotting. Normal bloods, both human and dog, and a group of slowly clotting specimens—canine hemophilic blood, human platelet-poor plasma and normal blood clotting in silicone-treated containers—were studied.

Materials and methods. Three normal human subjects, 6 normal kennel dogs and 3 hemophilic dogs(6) were used in these experiments. Blood was collected by venipuncture in 2 dry syringes. Four ml of blood from the first syringe were mixed with 0.5 ml 3.2% sodium citrate solution and the control plasma obtained by centrifugation. Blood from the second syringe was distributed immediately into a series of 10x75 mm dry glass tubes calibrated to 2 ml (28°C). Periodically, 0.25 ml of 3.2% sodium citrate was added to each tube and the citrated serum (or plasma) obtained by centrifugation (about 3000 g for 3 minutes). For the silicone experiments, silicone-treated needles, syringes and calibrated tubes were used(7). Platelet-poor human plasma was obtained as described previously(7). One-stage and 2-stage pro-

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

† Markle Scholar in Medical Science.

1. Schmidt, A., *Zur Blutlehre*, F.C.W. Vogel, Leipzig, 1892.

2. Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, v114, 667.

3. Brinkhous, K. M., *Am. J. Med. Sci.*, 1939, v198, 509.

4. Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 117.

5. Quick, A. J., *Am. J. Med. Sci.*, 1947, v214, 272.

6. Graham, J. B., Buckwalter, J. A., Hartley, L. J., and Brinkhous, K. M., *J. Exp. Med.*, 1949, v90, 97.

7. Buckwalter, J. A., Blythe, W. B., and Brinkhous, K. M., *Am. J. Physiol.*, 1949, v159, 316.