Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 74 MAY, 1950 No. 1

SECTION MEETINGS

MINNESOTA
Mayo Clinic, Rochester, Minn. March 31, 1950

MISSOURI
St. Louis University March 22, 1950

PACIFIC COAST
Stanford University April 8, 1950

Prerequisites of Successful Heparinization to Prevent Gangrene After Frostbite.* (17790)

KURT LANGE, LINN J. BOYD, AND DAVID WEINER. (Introduced by A. L. Copley.)
From the Department of Medicine, the Research Unit, New York Medical College.

Lange and Boyd(1) demonstrated by use of the fluorescein method, that gangrene after frostbite is due to a tremendous increase in capillary permeability which leads to loss of plasma from the blood stream into the tissue so that the red cells silt up the capillaries due to loss of suspending fluid. This red cell sludge solidifies and is finally converted into hyaline thrombi. These masses of agglutinated red cells obstruct the blood stream locally and lead to the gangrene of the dependent tissue. Lange and Boyd(1) first described that heparinization shortly after exposure and to the extent that coagulation time (Lee-White method) is never shorter than 22 minutes, prevented occurrence of gangrene in 82% of frostbitten rabbit legs. Under these conditions the red cell sludge does not become compact nor adherent to the wall but redissolves. Laufmann *et al.*(2) confirmed our findings in an entirely different set-up. He stated that red cell sludge is not transformed into masses adhering to the vascular wall when sufficient amounts of heparin are given. The necessity for an uninterrupted and frequently checked maintenance of prolonged coagulation time to prevent gangrene has been stressed by us repeatedly(3,4).

Quintanilla *et al.*(6) were not able to prevent gangrene after frostbite in rabbits, but they continued their treatment for 36 hours only, without check on coagulation time and

* Carried out under grants from the John and Mary R. Markle Foundation and later under contracts among the Office of Scientific Research and Development of the National Research Council and the Office of the Surgeon General, U. S. Army, and the New York Medical College.

1. Lange, K., and Boyd, L. J., *Surg., Gynec., and Obst.*, 1945, v80, 346.

2. Laufmann, H., Martin, W. B., and Tanturi, C., *Science*, 1948, v108, 283.

3. Lange, K., Weiner, D., and Boyd, L. J., *Am. Heart J.*, 1948, v35, 238.

4. Lange, K., Weiner, D., and Boyd, L. J., *New England J. Med.*, 1947, v237, 383.

6. Quintanilla, R., Krusen, F. H., and Essex, H. E., *Am. J. Physiol.*, 1947, v149, 149.

with apparently insufficient doses. They tried to prevent the sludge formation by heparin injections. Sludging, however, is not prevented by heparin at all as shown by Laufmann(2).

Lempke and Shumacker(7) showed that in mice whose tails were exposed to frostbite, a dose of heparin every 3 hours for 3 days after exposure completely prevented gangrene in 39% of the animals. Another 39% showed minimal lesions. The control group showed only 4.8% recovery. If the interval between the heparin injections, however, was extended to 4 hours, the loss of tissue was almost 3 times as great as in the group receiving heparin at shorter intervals. No animals survived without gangrene. After 3 hours, the coagulation time of most of the animals was still markedly elevated, while after 4 hours, the majority of them had returned close to normal. This illustrates that good results can be obtained only if the coagulation time is not permitted to return close to normal.

Fuhrman and Crismon(8) have attempted to prevent gangrene subsequent to frostbite in 3 rabbits by injecting 10 mg/kg of heparin for 3 days at 24, 12 or 8 hour intervals respectively. This dosage is completely insufficient as shown by Shumacker(9) as well as by us. The results obtained are, therefore, without significance.

Lewis(10) demonstrated that by giving heparin initially and following up with dicumarol, he was able to save 60% of the tissue that sloughed off in the controls. No data about the dosage are available. It is difficult to say whether it was sufficient to produce a continuous and ample prolongation of coagulation or prothrombin time respectively.

Most recently, Pichotka and Lewis(11) concluded that the use of heparin in frostbite is of no value. In a previous personal communication, Pichotka had told us that he has been unable to produce a continuous prolongation of coagulation time by the dosage we used. We are not able to explain these differences. Different strains of animals, feeding and housing conditions, are important. The dose used is unimportant, however, as long as a sufficient prolongation of coagulation time is maintained for 6 days after exposure. Pichotka and Lewis state that they could not prolong the coagulation time in their animals continuously to the desired level. Their results are, therefore, of value only in the respect that they show that insufficient heparinization does not prevent gangrene after frostbite. It is interesting to analyze in detail, however, the results of Pichotka and Lewis. In their Table I, a group of 5 surviving animals received 20 mg of heparin every 4 hours after frostbite. Athough this dosage arrangement cannot be considered satisfactory according to their statements, complete necrosis occurred only in 40% of the treated animals while the controls showed complete necrosis in 79% of the animals. The other groups with longer intervals between the injections failed to show any benefit. It is astonishing to note that these facts have escaped the attention of the authors and their statisticians. The group which has the best, although not satisfactory heparinization, comprises only 12 animals while the groups with completely unsatisfactory heparinization comprise 30 and 39 animals respectively. All 3 groups are then lumped together and failure of the treatment is concluded from the average. In another paper(12), the same authors report that of 42 insufficiently heparinized animals, 76% showed no secondary infections while the non-heparinized group of 36 animals shows absence of infection in only 44% of the cases after frostbite exposure. The authors state that they are unable to explain this difference, but do not wish to attribute it to heparinization.

In order to stress the importance of a *continuous satisfactory elevation of coagulation*

7. Lempke, R. E., and Shumacker, H. B., *Yale J. Biol. and Med.*, 1949, v21, 321; Bull. Cold Injuries and Hypothermia, National Research Council, Conf. on Cold Injuries and Hypothermia, 1948.

8. Fuhrman, F. A., and Crismon, J. M., *Clin. Invest.*, 1948, v27, 364.

9. Shumacker, H. B., *Surgery*, 1947, v22, 900.

10. Lewis, R. B., personal communication.

11. Pichotka, J., and Lewis, R. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 130.

12. Pichotka, J., and Lewis, R. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 127.

TABLE I.
Incidence of Gangrene After Frostbite in Relation to Prolongation of Coagulation Time.

Therapy	No. of animals	Complete recovery		Extensive partial or complete gangrene	
		No.	%	No.	%
Sufficient amt of heparin to keep coagulation time continuously above 30 min. for at least 5 days after exposure	34	29	85	5	15
Heparin but with occasional or common dips of coagulation time below 30 min.	17	4	24	13	76
None (controls)	32	—	—	32	100

time to prevent gangrene after frostbite, the following findings are presented.

One hind leg of each of 83 rabbits was immersed in an alcohol dry ice bath at −12°C to −30°C for 30 to 45 minutes using the technic described by us(1,4,5). Fifty-one animals were heparinized and 32 served as controls. The strain of the rabbits varied widely throughout the experiments. Small albino rabbits as used in the Friedman test were used initially and found to require extremely small amounts of heparin to maintain a sufficiently elevated coagulation time. Later, mixed strains of rabbits were used requiring widely varying amounts of heparin to obtain the desired prolongation of coagulation time. Finally, rabbits from one breeder of the Blue Vienna strain were used, giving fairly consistent results with a standardized dose of heparin. While 14 (67%) of these 21 animals showed an elevation of the coagulation time to at least 30 minutes (Lee-White method) with a standard dose immediately before the next injection, 7 (33%) fell short of this goal at one, or more frequently, several occasions on the same dose. While this is given as an example, we shall refrain from quoting doses of heparin since by injecting similar doses, similar effects on the coagulation time are not necessarily obtained. The amount of heparin must be individualized in each animal by variation of dose or preferably by variation of time interval between injections. Heparinization was accomplished by individual injections into the ear vein in 44 animals. The actual number of animals started on heparinization by repeated intravenous injections after exposure was 55. Eleven died from severe hemorrhages before the fate of the exposed leg could be determined with certainty. To avoid this difficulty (which does not exist in treatment of human cases by continuous intravenous drip) 7 rabbits were kept on a continuous intravenous drip into the ear vein while sitting in the conventional rabbit box and having one ear fixed to a splint attached upright to the upper border of the box. The flow was adjusted to a very small number of drops per minute. A feeder and a watering device were attached to the box. The rear end of the floor of the box consisted of wire mesh heavily covered with sawdust to permit drainage of urine. Only female rabbits were used in this group. Each day, the animals were taken out of the box for ½-hour, interrupting the infusion to permit revision of the dressing and to prevent contractures.

Exposures were carried out under light pentothal sodium anesthesia since the animals fight violently when exposed without anesthesia. It is difficult to understand how Pichotka and Lewis(11) were able to expose rabbits in an alcohol bath without anesthesia, since great force is needed to restrain the limbs thus producing strong local pressure and a violent alarm reaction with the probable secretion of large amounts of adrenalin. This obviously should be avoided under all circumstances.

5. Friedman, N. B., Lange, K., and Weiner, D., *Am. J. Med. Science*, 1947, v213, 61.

All 32 control animals (exposed exactly like the heparinized animals) showed gangrene to the upper border of the exposed area. Nineteen animals were sacrificed between the 4th and 6th day to obtain specimens for histologic studies. They showed, however, clear-cut signs of incipient gangrene and the histologic studies bore out this observation. Of the 51 heparinized animals, 34 had a coagulation time of 30 minutes or more immediately before the next injection was given or, in those on the continuous intravenous drip, twice a day (Table I). Seventeen showed at one or several occasions a coagulation time below 30 minutes or even within the normal range.

Of the 34 animals showing a satisfactory elevation of coagulation time for at least 5 days, 5 developed complete or extensive partial gangrene (15%). Of the 17 animals showing a coagulation time below 30 min. at one or several occasions, 13 developed complete or extensive partial gangrene (76%).

It is thus evident that a continuous satisfactory elevation of the coagulation time of various duration depending on the severity of the lesion is a prerequisite for the successful prevention of gangrene after frostbite. Experiments in which this is not achieved are of no value in judging the merits of the method.

We suggest that the term "heparinization" be reserved only for such applications of heparin therapy where there is proof of a continuously maintained elevation of the coagulation time. The term should not be used for cases in which heparin is injected but proof of a satisfactory result is missing.

Summary. 1. Prevention of gangrene after severe frostbite requires a *continuous uninterrupted* prolongation of the coagulation time by heparin injections or infusions for at least 5 days after the exposure.

2. Even brief interruptions of this prolongation lead to a rapid increase in percentage of failure of the treatment.

3. Experiments in which such a prolongation is not achieved continuously are of no value in judging the merits of the therapy.

4. The term "heparinization" should be reserved for such instances in which heparin injections are proved to have produced a continuously maintained elevation of coagulation time to the desired level.

Received January 20, 1950. P.S.E.B.M., 1950, v74.

Determination of Small Quantities of Nitrogen in Serological Precipitates and Other Biological Materials.* (17791)

FRANK LANNI, M. L. DILLON, AND J. W. BEARD.

From the Department of Surgery, Duke University School of Medicine, Durham, N. C.

There exists a need for a simple and accurate method for the analysis of nitrogen in microquantities of protein, with particular application to serological precipitates. Pressman(1) and Heidelberger and MacPherson(2) have described quantitative colorimetric methods which employ the Folin-Ciocaltéu phenol reagent(3) and require an empirical calibration curve for converting the phenol color readings to nitrogen or protein. The precision and accuracy of these methods can be readily obtained by the use of Nessler's reagent, with which nitrogen is measured directly as ammonia. The method described below has been employed successfully in this laboratory for the past two years, and certain

* This work was supported by a grant to Duke University from Lederle Laboratories, Inc., Pearl River, N. Y. and in part by the Commission on Influenza, Army Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

1. Pressman, D., *Ind. Eng. Chem., Anal. Ed.*, 1943, v15, 357.

2. Heidelberger, M., and MacPherson, C. F. C., *Science*, 1943, v97, 405; v98, 63.

3. Folin, O., and Ciocaltéu, V., *J. Biol. Chem.*, 1927, v73, 627.