Shortening of Bleeding Time by a Water-soluble Adrenochrome Derivative.* (18343)

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Oxidation and other chemical modifications of the epinephrine molecule affect its various physiological properties in varying degree and sense. Oxidative ring closure which leads from epinephrine to adrenochrome converts a pressor into its opposite, an antipressor substance(1). The hemostatic effect of epinephrine is altered in degree, but not in sense by the same chemical change(2). The property of adrenochrome of shortening the bleeding time is shared by adrenalone, a less profoundly modified oxidation product of epinephrine(3).

Because of the limited solubility and of the instability of adrenochrome in solution, one of us has prepared a series of highly water-soluble adrenochrome derivatives which will be described elsewhere(4). The observations of Derouaux that adrenochrome shortens the bleeding time in rabbits and subsequent reports(5) that similar effects could be obtained with the monoxime, monosemicarbazone and mono-dinitro-phenylhydrazone of adrenochrome suggested a study of the hemostatic effect of a compound representing our series of water-soluble adrenochrome derivatives. We selected the trimethylammoniumacethydrazone hydrochloride of L-adrenochrome (T.A.L.). Observation of a shortened bleeding time upon administration of this compound would indicate that the chemical modification of the parent substance adrenochrome in this type of derivative does not impair one of its physiological effects.

Material and methods. Trimethylammoniumacethydrazone hydrochloride of L-adrenochrome is a red substance melting with decomposition at 160°; it is extremely soluble in water. The finely ground substance was mixed with 4% of its weight of anhydrous disodium phosphate and distributed in the desired dosage, usually 10 mg, in sterile ampules. The contents were dissolved in sterile distilled water immediately before use, giving a solution of pH 6.5. The bleeding time was determined by the method of Ivy (6). A blood pressure cuff is placed around the arm and inflated to 40 mm Hg pressure. The cleaned skin of the forearm is pricked with an automatic lancet. The drop of blood which collects at this site is removed every 10 seconds by blotting paper; this permits the determination of the bleeding time within 5 to 10 seconds.

Results. In a group of preliminary tests, amounts up to 2 mg were administered by Dr. S. E. Moolten at St. Peter's Hospital in New Brunswick, N. J., to a number of patients by intramuscular injection and in a few cases *per os*. The bleeding time was determined before and about 1 hour after the medication. In two-thirds of the cases the bleeding time was diminished after administration of the compound and neither local reactions nor any other untoward symptoms were seen. On the basis of these preliminary observations we felt encouraged to study a larger series. A group of 50 consecutively admitted children, between the ages of 2 and 16, undergoing tonsillectomy and adenoidectomy were studied with the proper controls to exclude the effects of simultaneous surgical procedures. Of the 50 subjects two-thirds were injected

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2. Derouaux, G., *Arch. int. Pharmacodyn. et Thér.*, 1941, v66, 202.

3. Derouaux, G., *Arch. int. Pharmacodyn. et Thér.*, 1939, v62, 1939.

4. Sobotka, H., and Austin, J., *Betaine Hydrazones of Aminochromes,* in press.

5. Derouaux, G., *Arch. int. Pharmacodyn. et Thér.*, 1943, v69, 142.

6. Kracke, R. R., *Diseases of the Blood,* Lippincott, Phila., 2nd Ed., p.636, 1941.

intramuscularly with 10 mg of T.A.L. dissolved in 1 ml of water, while one out of 3 served as control. The injection was administered immediately before removal of the patient to the operating room. In view of the nature of the operation no clinically noticeable effects were to be expected. No reactions of any sort were noted in the children receiving the medication. There was no unusual change in the pulse. The amount of bleeding in these children during the course of the tonsillectomy and the adenoidectomy appeared about the same as in the control group. No blanching or other changes in the mucous membranes of the pharynx were noted. There was no post-operative hemorrhage in either control or the treated group. There were no local reactions at the site of the injection.

The bleeding time was again taken after the patient had returned from the operating room and recovered from the anesthesia. The time interval between the two determinations varied from 3½ to 6 hrs and averaged 4 hrs 35 min for the treated cases and 4 hrs 30 min for the controls. Whether a patient had received medication or not, remained unknown to the person who measured the bleeding time.

As shown in Fig. 1 the bleeding time was found reduced in 25 out of 32 cases to values as low as 22% of the original

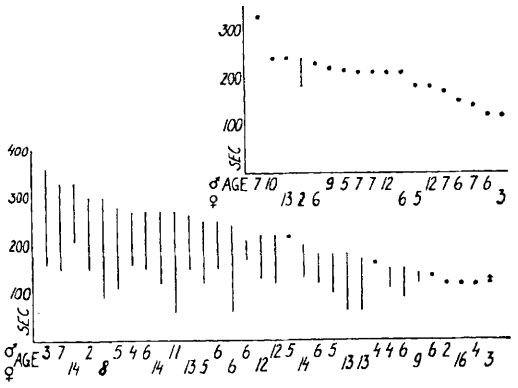

FIG. 1.

Shortening of bleeding time in a group of 32 children upon administration of 10 mg T.A.L. The vertical lines connect the point which indicates pre-injection bleeding time with the point giving the bleeding time after an average period of 4½ hr following the injection. The bleeding time in non-responsive cases is indicated by dots.

bleeding time. In 6 cases there was no difference between the bleeding time before and after administration of T.A.L. and in one case an insignificant lengthening. The average shortening of the bleeding time was 38% for all cases, but 49% for those 25 cases which showed any response at all. It is noteworthy that the pre-injection bleeding time of the responsive group averaged 4 min (238 sec) as against 141 sec for the 7 non-responsive individuals. This indicates that the hemostatic mechanism, upon which T.A.L. acts, hardly permits of further enhancement in cases with an initial bleeding time of 2 min. No correlation was observed between the response and the age and sex of the patients.

In the control group of 18 non-treated children with a similar distribution of age and sex, the bleeding time varied individually from 120 to 330 sec (See inset, Fig. 1). The median bleeding time of this group was identical with that in the injected group, namely 210 sec. The average bleeding time of the controls was 201 sec in satisfactory agreement with 218 sec, the overall average of the injected group. The bleeding time of the control cases remained remarkably constant before and after the surgical intervention; in 17 cases the bleeding time was identical within 5 sec as measured before and after the operation. In only one control case, a drop of bleeding time from 240 to 180 sec was encountered. It was noted in several cases that the bleeding time had returned to its original value when they were retested on the third day after an effective injection. Likewise the bleeding time of several control cases proved identical when retested 3 days later.

Discussion. The mechanism of the hemostatic effects of sympatomimetic compounds and of their oxidation products has been studied and discussed in numerous publications by Roskam of Liège and his school(2,3,5). Other physiological effects of these products have been investigated by Bacq(7). Oster(8) has dealt with the mechanism of their antipressor

7. Bacq, Z. M., *cf. Presse méd.*, 1947, v16, 175.
8. Oster, K. A., *Nature*, 1942, v150, 289.

action. The therapeutic exploitation of these various pharmacological properties requires the appropriate form for administration. New compounds, synthesized by modification of chemical functional groups with a view to increased stability and solubility, must be compared with the parent substance in respect to the perseverance of the desired activity. In the present instance, we have converted adrenochrome into the stable and extremely water-soluble betaine hydrazone T.A.L. The clinical tests which we report show that the formation of the derivative has not destroyed the hemostatic effects of adrenochrome. These results, in addition to their practical interest *per se*, may serve as a guide in the study of the metabolic fate of the compounds; moreover, they point to the localization of the hemostatic effect in that part of the molecule not affected by the synthetic chemical modification.

Summary. Trimethylammoniumacethydrazone hydrochloride of L-adrenochrome (T.A.L.) is a potent hemostatic agent. It shares this property with the parent substance adrenochrome, but surpasses it by stability and solubility in aqueous solution. It may be injected intramuscularly in children in amounts of 10 mg without producing local reactions. A single injection of this amount shortens the bleeding time, as measured by Ivy's method, by an average of 38% in 32 subjects; except for 7 non-responsive cases with short pre-injection bleeding time, the mean bleeding time in the remaining 25 cases is cut in half. Only individuals with a short bleeding time fail to respond. Control tests show that the concomitant surgical intervention has no influence on bleeding time.

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Effect of Maleic Acid on Renal Function. (18344)

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As part of an investigation of the capacity of certain organic acids to serve as effective buffers for the excretion of titratable acid, maleic acid was selected since its pK (about 6.5) is such as to be theoretically well suited to this function. It was observed, however, that when maleic acid was administered to an acidotic dog, the urine became alkaline despite the fact that the animal was in severe acidosis (plasma pH 7.0, CO_2 content 9.5 mM/1). Further investigations of the effects of maleate on renal function are the basis of the present report.

Methods and material. Trained, unanesthetized female dogs in the post-absorptive state were used in all experiments. Acidosis was induced by the administration of 5 g of NH_4Cl eighteen hours before some of the experiments and in others by the inclusion of dilute HCl in the infusion fluid. The technics and chemical methods used have been described in earlier publications from this laboratory(1,2). Maleate, generally at a dosage of 40 mg (0.35 mM) per kilo, was administered intravenously as a single dose in all experiments. The pH of the injected material

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