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Anti-Coagulant Properties of Ethylene Bis-iminodiacetic Acid. (18577)

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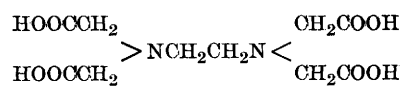
The ethylene bis-iminodiacetic acid (Synonym: ethylenediamine tetra-acetic acid) a polyaminocarboxylic acid derivative* is one of the most powerful deionizing agents for alkaline earth and metals. It is available as the free acid, and as di-, tri- and tetrasodium salt, under the trade name of Sequestrene. The structural formula of the free acid is as follows:



The free acid is only slightly soluble in water, while the sodium salts are readily soluble. The free acid and its salt do not hydrolyze in aqueous solution, and are stable and effective over a wide range of temperatures and pH.

Dyckerhoff(1), *et al.*, were the first to investigate the anti-coagulant effect of the di-sodium salts of Sequestrene. They found that one half to one mg completely inhibited the coagulation of human and animal blood, and that it was 10 times more effective than sodium citrate. The anti-coagulant effect is due to the binding of the blood calcium ion. It has no effect on blood clotting *in vivo*. The di-sodium Sequestrene has been used in our laboratory for routine blood work for over a year. Because of its powerful anti-coagulant properties, only minimum quantities are necessary. It is less damaging to

* The ethylenediamine tetra-acetic acid was first synthesized by the I. G. Farben Industry in Germany. It is now manufactured in the U.S.A. by the Alrose Chemical Co., Providence, R. I., under the trade name of Sequestrene. Four Sequestrenes are available at present, Sequestrene AA (the free acid), SNA 2 (di-sodium salt), SNA 3 (trisodium salt) and SNA 4 (tetrasodium salt).

1. Dyckerhoff, Marx, and Ludwig., *Zt. f. d. ges. exp. Med.*, 1944, v110, 412.

the cellular elements of the blood, and preserves them longer than sodium citrate and the oxalates. For routine blood examination, a 10% aqueous solution (maximum solubility) is preferable; 0.005-0.01 cc will keep 5 cc of blood from coagulating.

If blood is kept at 5°C, the red cell and leucocyte counts do not show any significant changes for from 3-5 days. If blood is collected in paraffinized containers, the platelet count remains unchanged for 24 hours; after this time a slight decrease is noted (6%). After the initial drop, there is no appreciable change in the count for about 4 days, as compared with a decrease of about 50% in sodium citrate. Hemoglobin, bilirubin and sedimentation speed remain unchanged for several days, if the blood is kept at 5°C and not shaken excessively. The stainability for the cellular elements remains unchanged for a week or longer.

The agglutinogens of the red cells and the oxydase reaction of granulocytes are not destroyed by Sequestrene NA 2. The red cells are strongly agglutinated by their specific antisera, and the oxydase reaction remains positive after blood has been preserved for 3 weeks at 4-5°C.

When given *per os,* Sequestrene NA 2 is practically atoxic for mammals. Rats fed maximum doses did not show any toxic symptoms except diarrhea. Ninety-six per cent of the administered Sequestrene was excreted unchanged in the feces of rats fed massive single doses. The coagulation time was not altered significantly, and no pathological changes in the cellular elements of the blood were noted.

Dyckerhoff found that rabbits tolerated parenteral injection of as much as 80 mg of ethylenediamine tetra-acetic acid per kg, without toxic symptoms. When 100 mg per kilo were given, the animals developed tetany, but recovered rapidly upon administration of calcium ion. Rabbits tolerated intravenous injections of 40-80 mg per kg daily for 10 days. When given intravenously, Martin(2) found 50 mg toxic for dogs. The animals recovered when injected with 100 mg of calcium gluconate. Sequestrene was well tolerated when injected simultaneously with 100 mg of calcium gluconate.

Preliminary experiments for substituting di-sodium Sequestrene for sodium citrate in preserving human blood, have shown that it preserves blood longer if kept at 4-5°C. 1.5 g di-sodium Sequestrene dissolved in 100 cc of 5% glucose, will keep 400-500 cc of blood from coagulating. The di-sodium salt does not caramelize when sterilized with glucose; the sterilized solution has a pH around 5. Twenty human transfusions with blood preserved in the above solution, did not show any ill effects. No tetany, or changes in the coagulation time were noted. Further transfusion experiments are in progress and will be reported later.

2. Martin, G. I., *Meeting A.C.S.,* 1949 Abstracts, 117, 16C.

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Mechanism of Adrenal Involution in the Rat After Treatment with Thiouracil. (18578)

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Previous work has shown that prolonged treatment with thiouracil will produce atrophy and damage of the adrenal cortex(1-3). In the rat, this decrease in the weight of the

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1. Bauman, E. J. and Marine, D., *Endocrinology,* 1945, v36, 400.

2. McQuillan, M. T. and Trikojus, V. M., *Brit. J. Exp. Path.,* 1946, v27, 247.