Enhancement of Jejunal Absorption of Heparinoid by Sodium Ethylenediaminetetraacetate in the Dog.* (27900)

C. S. TIDBALL† AND R. I. LIPMAN (Introduced by C. E. Leese)
Department of Physiology, George Washington University School of Medicine, Washington, D.C.

The intestinal absorption of weak organic acids of molecular weight in excess of 200 is dependent on their ability to dissolve in the lipoid matrix of the mucosal membrane(1). The pH of the luminal solution becomes a limiting variable as it shifts the equilibrium of the dissociation toward greater ionization since the ionic forms of the weak acids are considerably less lipid soluble than the non-ionized molecules. Thus as Loomis(2) has shown, sodium heparin is absorbed from canine duodenum to a small extent at pH 4 but not at higher pH. This may be due to the protonization of the glucuronic acid carboxyl groups of heparin reducing ionization of the complex at lower pH. Such a process has been suggested for the glucuronic acid carboxyl groups of chondroitin sulfate by Warner and Schubert(3). Utilizing these concepts Salafsky and Loomis(4) altered the structure of heparin by esterification and produced a heparinoid that retained anti-coagulant action and was significantly absorbed by canine duodenum from solutions at physiologic pH. An alternate approach was that of Windsor and Cronheim(5) who added sodium ethylenediaminetetraacetate (NaEDTA) to a different heparinoid and were able to demonstrate enhanced intestinal absorption following oral administration of the combination.

The present report is a reexamination of the ability of NaEDTA to enhance intestinal absorption of heparinoid under improved experimental conditions. Lipemia clearing activity was found inadequate to follow repeated administrations of heparinoid making it unsuitable for experiments where measurements are made before and after the presence of NaEDTA in the intestine. A sensitive plasma clotting time method overcame this difficulty and permitted the statistically advantageous use of an animal as his own control. Absorption occurred from an isolated jejunal segment *in situ* which made direct measurement of the extent of absorption possible. Both indirect and direct means verified that NaEDTA was able to increase the intestinal absorption of heparinoid.

Methods. Experiments were carried out on dogs anesthetized with intravenous sodium pentobarbital (30 mg/kg). A 25 cm segment of jejunum was isolated from the remainder of the tract by soft string ligatures and glass cannulae were tied into each end. The loop was flushed with physiologic saline and returned to the peritoneal cavity before the start of the absorption periods which were one hour in length. All solutions placed in the lumen were adjusted to pH 7. The isolated segment was filled with a balanced physiologic saline containing 10 mg/ml of S^{35} labelled heparinoid‡ from a previously weighed reservoir system at 10 cm hydrostatic pressure, and the system closed off. At 15 minute intervals the stopcock was opened and the loop allowed to refill. The system was again weighed at the end of the hour and the difference in weight in grams was taken as the milliliters of test solution that had entered the loop. The isolated segment was then opened at its other end and its contents washed into a collecting vessel with 500 ml of physiologic saline not containing heparinoid. This control period was followed by an identical experimental period in which the only difference in procedure was the addition of 25 mM NaEDTA to the test solution. The presence of mucin and other materials from the passage of the fluid through the intestinal loop did not alter the

* This work was supported by a grant from Riker Laboratories, Northridge, Calif.

† Work performed during tenure of U. S. Public Health Service Special Fellowship in Gastroentrology.

‡ The carrier and S^{35} labelled heparinoid were provided by Dr. Louis Levy, Riker Laboratories, Inc., Northridge, Calif.

radioactivity of plated samples; however the concentration of heparinoid did alter the plating and for this reason all samples were plated at similar heparinoid concentrations.

Venous blood for clotting time and lipemia clearing activity determinations was collected in a syringe moistened with 200 mM NaCitrate, and 4.5 ml of whole blood were promptly added to 0.5 ml of the same NaCitrate. After gentle mixing the blood was centrifuged at 1000 RPM for 6 minutes. The plasma was separated and well mixed before aliquots were taken for determination of clotting time. This step is essential to insure reproducibility of repeated determinations. One-half ml of the mixed plasma was brought to 37°C after which an equal volume of 25 mM $CaCl_2$ was added and a stopwatch started. A sharp end point was obtained when fibrin strands became visible. This occurred in approximately 30 seconds for normal blood. A stable base line of clotting time was obtained before initiating either control or experimental periods. Lipemia clearing was determined by the turbidimetric method of Grossman(6).

Results. Preliminary experiments with single intravenous injections of heparin and heparinoid definitely elevated the lipemia clearing activity to values as high as 6 Grossman Clearing Units. However, when quantities of heparinoid were infused intravenously over one hour, so that the hourly dose was equal to the single injection, no change in clearing activity was noted. By increasing the dose, changes were obtained; but a second intravenous infusion, after a return of the clearing activity to control levels, did not produce the same response. Doubling the amount of heparinoid infused in the second hourly period failed to produce as great a change in the clearing activity as was seen in the first hourly period of infusion. Further, the amount of clearing activity obtainable from standard amounts of heparinoid was not reproducible from animal to animal. Accordingly, a clotting time technic was substituted to measure the arrival of heparinoid into the bloodstream.

In 11 control absorption periods without NaEDTA present an average of 10 mg heparinoid was absorbed per hour (Table I). During 9 experimental periods with 25 mM NaEDTA present an average of 88 mg heparinoid was absorbed per hour. The difference between control and experimental absorption periods for each animal is given in the fourth column and is the basis for a statistical evaluation which indicated that the difference is significant at the 98% confidence level. Clotting time information was obtained on 4 of these experiments, 2 of which are shown in Fig: 1. With the control absorption a small change or no change was seen in clotting time. The interval be-

TABLE I. Absorption of Heparinoid with and without NaEDTA.

	Absorption of heparinoid in mg/hr		
Exp. No.	Control (without NaEDTA)	Experimental (25 mM/l NaEDTA)	Difference (exp − control)
1	4		
1A	0		
2	3	57	54
3	17	59	42
4	− 1	248*	249
5	44	132	88
6	−20	71	91
7	1	54	53
8	6	33	27
9	13	59	46
10	44	81	37
Mean ± S.E.	10 ± 6	88 ± 22	76 ± 23†

* Return from loop blood tinged.

† $t = \frac{\bar{d} - 0}{s/\sqrt{N}} = 3.36$; $t_{0.99}$ (8 D.F.) = 2.90.

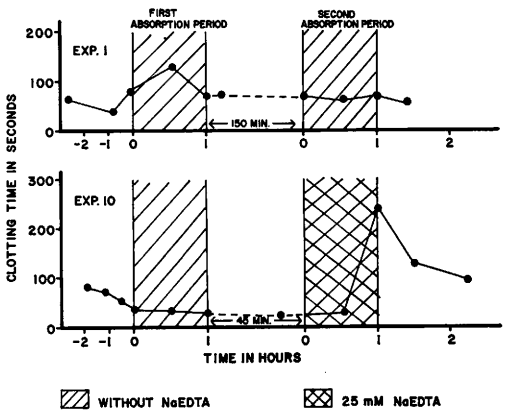

FIG. 1. Clotting time curves during heparinoid absorption with and without NaEDTA.

tween the first and second absorption periods, indicated in the column between the 2 periods, was prolonged as necessary to obtain a stable clotting time curve. Clotting times were uniformly elevated in the 3 NaEDTA experiments. The maximum response in Exp. 10 consisted of a 5-fold increase in clotting time.

Discussion. These results confirm the observations of Windsor and Cronheim on the ability of NaEDTA to enhance intestinal absorption of heparinoid without altering the molecule to such an extent that it loses its anticoagulant properties. NaEDTA has also been shown to increase the absorption of phenolsulfonphthalein, a strong acid of moderate molecular dimensions(7). According to Shanker and Johnson(8) the effect of NaEDTA is a general one which will enhance absorption of basic and neutral compounds as well as acidic ones. The movement of inulin from blood to intestinal lumen was also shown by them to increase 4-fold when NaEDTA is present in the intestinal lumen. NaEDTA was found by Curan *et al.*(9) to increase the conductance of the isolated frog skin while both short circuit current and the potential difference across the skin decreased. These results are consistent with a generalized increase in permeability of the frog skin. The reversibility of these findings upon addition of $CaCl_2$ in the frog skin and the lack of histological evidence of damage to canine mucosal epithelium (unpublished observations from this laboratory) argue against a permanent dissolution of the membrane. The reversibility with $CaCl_2$ in the frog skin coupled with the observations of Cronheim and Windsor that CaEDTA did not produce the increased absorption, suggest that removal of Ca^{++} from the membrane is of primary importance in producing this change in permeability. The occasional bloody return in our experiments and the report of intestinal hemorrhages without lesions in a case of NaEDTA therapy for hypercalcemia which came to autopsy(10) provide a warning that there may be limits beyond which the permeability of the intestinal epithelium should not be increased.

Summary. The presence of NaEDTA in the lumen of isolated canine jejunal segments *in situ* produced significant enhancement of intestinal absorption of heparinoid as measured by the disappearance of heparinoid from the lumen. Increases in clotting time of recalcified citrated plasma accompanied the enhanced absorption and verified that the heparinoid had been absorbed without loss of anticoagulant activity.

1. Wilson, T. H., *Intestinal Absorption,* Saunders, Philadelphia, 1962, p44.

2. Loomis, T. S., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 447.

3. Warner, R. C., Schubert, M., *J. Am. Chem. Soc.,* 1958, v80, 5166.

4. Salafsky, B., Loomis, T. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 62.

5. Windsor, E., Cronheim, G. E., *Nature,* 1961, v190, 263.

6. Grossman, M. I., *J. Lab. Clin. Med.,* 1954, v43, 445.

7. Tidball, C. S., Peterson, K. K., *The Physiologist,* 1961, v4, No. 3, 121.

8. Shanker, L. S., Johnson, J. M., *Biochem. Pharmacol.,* 1961, v8, 421.

9. Curran, P. F., Zadunaisky, J., Gill, J. R., Jr., *Biochim. Biophys. Acta,* 1961, v42, 392.

10. Dudley, H. R., Ritchie, A. C., Schilling, A., Baker, W. H., *New Eng. J. Med.,* 1955, v252, 331.

Received August 16, 1962. P.S.E.B.M., 1962, v111.