Fried and Borman speculated on possible role of 9α-halo modifications in glucocorticoids (2). Data were presented to demonstrate parallelism between glucocorticoid activity and electronegativity, or ability of 9α-halo substituents to strengthen acidity of adjacent 11β-hydroxy radicals in steroids. However, that 9α-F-DCA is 12-14 times as potent as DCA would suggest other factors besides the influence of electronegativity must account for increased mineralocorticoid effects with 9α-halogenation.

Summary. The mineralocorticoid effects of 9α-fluorodeoxycorticosterone acetate were investigated in adrenalectomized rats. A quantitative 4-point assay gave potency estimates of 12 and 14.3 for the steroid relative to DCA, as judged by reduction of urinary Na excretion and the ratio of Na K, respectively. Thus, addition of a 9α-fluoro radical can increase mineralocorticoid activity in a steroid lacking oxygen function at carbon 11. These and other published data suggest that 9α-halogenation plays a singularly important part in high DCA-like activity of various 9α-halo corticosteroids.

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Received March 3, 1960. P.S.E.B.M., 1960, v104.

Intestinal Absorption of a Modified Heparin.* (25727)

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Schanker *et al.*(1) and Hogben *et al.*(2) have shown that intestinal absorption of many drugs is in part dependent on the state of ionization of the drugs. Drugs which are weak organic acids or bases were more completely absorbed from the gastro-intestinal tract in pH ranges which favor the unionized radical. Loomis(3) has shown that sodium heparin is absorbed from the duodenal lumen of the dog at pH 4 but not at higher pH. Warner(4) believes that glucuronic acid carboxyl groups of chondroitin sulfate protonize between pH 5 and 2 thereby reducing ionization of the complex. A similar situation may exist for glucuronic acid carboxyl groups of heparin. If ionization is an important factor in regulating absorption from the intestine, then suitable modification of ionizing potentialities of heparin may enhance absorption in the physiological pH range. The current study is an attempt to accomplish this by esterification of heparin. A modified heparin has been prepared and evidence presented indicating that it is partially methylated and has undergone partial desulfation. It retains its original anticoagulant action and significant absorption

* This work supported by grant from Nat. Inst. of Health.

TABLE I. Elemental Analyses: Radioactive Samples Are Corrected for Background and Are Average of 2 Determinations.

	Carbon[14]	Sulfur		Sodium	
Sodium heparin		11.4%	S.D. .34	9.9%	S.D. .24
Unpurified heparin derivative	125c/m/30 mg	10.4	" .94	7.9	" .24
Purified heparin derivative	116c/m/30 mg	10.6	" .41		

from the intestine occurs at pH 5 and 6.

Methods. Powdered sodium heparin† was suspended in methanolic-HCl (96.5% methanol, 3.5% 1N HCl). The suspension was kept at room temperature 1 hour, then centrifuged. The supernatant was discarded and the precipitate dried in vacuum at 0°C. The product was then powdered and used without further purification in animal experiments. Additional identical preparations were made using C^{14} methanol and the precipitate purified by washing with methanol and by reprecipitation from aqueous solution by addition of alcohol to 70% by volume. The precipitate was then dried. Analysis for C^{14}, S(5), and Na(6) were made on the unpurified product and analyses for C^{14} and S were made on the purified product. *In vitro* Lee-White (7) clotting times were performed on the modified product and compared to sodium heparin standards. Fourteen adult mongrel dogs were fasted 24 hours. Under pentobarbital (30 mg/kg) intravenous anesthesia, 15 to 20 cm length of duodenum located about 20 cm from the pylorus was exposed and transected at both ends leaving mesenteric blood supply intact. This loop of intestine was irrigated with approximately 200 ml of physiological saline and the distal end ligated. Undiluted MacIlwaine buffer(8) (Phosphate-citric acid) solutions containing the modified heparin were instilled into the loop and proximal end ligated. The segment was replaced in the abdomen and the incision closed with clamps. Systemic anticoagulant effect was determined by Lee-White(7) 3-tube method on femoral venous blood samples before and at ½ or 1 hour intervals following instillation of the solution in intestinal loop.

Results. Table I shows that purified C^{14} methanolic-HCl treated preparation of heparin retained much of its C^{14} activity indicating partial methylation of heparin. Sulfur content of both purified and unpurified product was decreased approximately 11.0% in comparison to sodium heparin. Sodium content of the unpurified product decreased approximately 20% compared to sodium heparin. Fig. 1 indicates *in vitro* anticoagulant effect of modified heparin was equal to that of original sodium heparin over a range of 0.5-5 μg. Table II indicates that 200 mg of modified heparin instilled into intestine with 20 ml of buffer having pH values of 5, 6, and 7 resulted in various degrees of anticoagulant effect for 5 hours. In presence of these buffers a significant anticoagulant effect was generally obtained within the first half hour. Both intensity and duration of anticoagulant effect diminish with rise in pH. The pH of luminal contents at end of experiments was 6 to 6.5 regardless of buffer used. Intestinal loops showed no gross evidence of general mucosal inflammation; however, there was a local inflammatory response at and adjacent to the area of site of application of ligature at each end of loop.

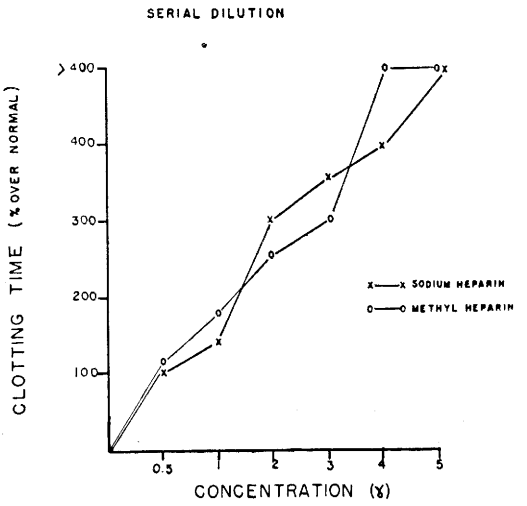

FIG. 1. Serial dilution of sodium heparin and modified heparin as measured by the Lee-White 3 tube method. Volumes in all tubes were 0.1 ml.

† Heparin was generously supplied by Eli Lilly and Co., Indianapolis.

TABLE II. Influence of Buffers at pH 5, 6, and 7 on Absorption of Modified Heparin Ester from Duodenal Loop of Dogs. Clotting time of venous blood recorded to nearest 10% of control. Each dog acted as its own control. Control clotting times were between 14 and 21 min. The number 0 indicates clotting time had fallen to, or below, control at that period. In dogs 1, 4, and 9 venous sampling was discontinued prior to cessation of anticoagulant effect. Figures of 400 represent values of 400% of control or above.

		Clotting time of blood as % of control										
	Buffer	Hr after instillation										
Dog No.	pH	½	1	1½	2	2½	3	3½	4	4½	5	5½
1	5	180	400	400	400		400					
2	5	150	"	"		400		400	400	220	90	0
3	5	260	"	"	"	390		230	110	0		
4	5	190	390	"		400						
5	6	110	120		0							
6	6	400	400	"	400				0			
7	6	180	"	"	"		310		40			
8	6	130	"	300	130	30	0					
9	6	0		400	400		400	400		400		
10	7	160	"		"	400	"	280		0		
11	7	170	350	230	10	0						
12	7	0	220	210	130	30	0					
13	7	No effect										
14	7	60	0									

Discussion. The current lack of conclusive information regarding the structure of heparins precludes specifically identifying alterations produced in the modified heparin we used. Wolfrom and co-workers(9) represent a heparin as a tetrasaccharide unit composed of alternating glucosamine and glucuronic sugars containing 5 sulfonic acid residues. Two of these sulfate residues are linked to the NH_2 group of glucosamine. The other 3 are distributed as O-sulfate residues. Sodium heparin would be represented by one sodium atom/sulfate residue. Mild acid hydrolysis (10) involves cleavage of the labile sulfamic acid groups. This may account for the observed decrease in sulfur and sodium in the preparation we used. The O-sulfate groups undergo slower hydrolysis(10) and would not be expected to hydrolyse under the mild conditions we used. Each glucuronic acid unit has a terminal, free, carboxyl radical. Wilander(11) attributes acidity of heparin to the carboxyl group in accordance with data derived from titration curves. If carboxyl groups of heparin protonize between pH 5 and 2, as in chondroitin sulfate, and thereby render the molecule less ionized, this may explain observed absorption of sodium heparin at pH 4 described by Loomis(3). Partial methylation of these carboxyl radicals might account for the observed absorption of this compound at a more physiological pH. Minimal desulfation may also play a role in enhancing absorption at pH 5 and 6. However, as pH approaches neutrality the modified heparin is less significantly absorbed.

Using Loomis's(12) detoxication value for sodium heparin of 5 μg/kg/min, only approximately 5% of instilled modified heparin was absorbed in current experiments. If the pH of luminal contents was the sole factor regulating absorption, since some absorption occurred at pH of 6 to 7, the anticoagulant should continue to be absorbed at this pH range until absorption is more nearly complete. However, the pH of luminal contents at the end of experiments was 6.0 to 6.5 when systemic anticoagulant effect was not evident. Current experiments permitted absorption only from the segment of intestine containing heparin. Experiments of Schanker involved perfusion of entire small intestine of the rat until equilibrium was reached and then only approximately ½ of many drugs was involved. It is possible that presence of buffer salt is important in influencing absorption of the modified heparin by a mechanism other than its buffering action. Following progressive absorption of the buffer salt total ion concentration in the intestinal loop would be expected to change. Prior experiments have shown that no anticoagulant effect occurs following instillation of only the MacIlwaine buffer of pH 4 and 7.

Summary. A modified heparin has been prepared. This heparin derivative has undergone slight methylation and partial de-sulfation. Anticoagulant activity was retained, and limited absorption from duodenum of dogs as measured by systemic anticoagulant effect occurred following instillation of the modified heparin in buffer solutions of pH 5, 6, and 7.

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Received March 3, 1960. P.S.E.B.M., 1960, v104.

Further Observations on Mechanism of Hypoglycorrhachia in Experimental Meningitis.* (25728)

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We have demonstrated that incubation of CSF containing polymorphonuclear leukocytes with pneumococci at 37°C resulted in greater consumption of glucose(1) than when organisms were incubated in cell-free CSF. CSF containing a comparable number of leukocytes in absence of bacteria displayed little glycolytic activity. These findings pointed to definite synergistic effect between polymorphonuclear leukocytes and bacteria in depressing CSF glucose. The reaction was dependent upon number of bacteria and white cells in the incubation mixture but was not altered when erythrocytes were added to the medium. The mechanism of synergistic action of leukocytes and bacteria in enhancing glycolysis in the CSF was not clear, but it was suggested that bacterial cells multiplied more rapidly in the presence of leukocytes resulting in increased consumption of glucose, or that white cells engaged in phagocytosis exhibit greater glycolytic activity. Evidence will be presented here that the synergistic effect between cells and bacteria in producing hypoglycorrhachia is a function of enhanced phagocytosis in the subarachnoid space of intact animals.

Methods. Aseptic meningitis was produced in mongrel dogs by injection of 4 ml of sterile pyrogen-free, 0.85% NaCl into the cisterna magna. Four hours later 4 ml of CSF which regularly contained 2000 to 4000 WBC/mm^3 (95-99% polymorphonuclear leukocytes) was removed by cisternal puncture. The animals were then given 4 ml of physiological saline containing 10^8 viable pneumococci intrathecally and 3 hours later CSF was again removed for quantitative determinations of bacteria, leukocytes and glucose. One group of control animals was given physiological saline at beginning of experiment and 4 hours later,

* Supported by contract with Army Chemical Corps, Fort Detrick, Md., and grants from Parke-Davis Co. and Upjohn Co.

† Work performed during tenure of Lederle Medical Faculty Award.