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Received December 9, 1958. P.S.E.B.M., 1959, v100.

Inactivation by Plasma of ACTH-Releasing Property of C^{14} Labeled Bacterial Polysaccharides.*† (24616)

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A marked, but transient, elevation of plasma 17-hydroxycorticosteroids (17-OHCS) follows intravenous injection of a polysaccharide complex from *K. pneumoniae* into the guinea pig(1.2). The plasma 17-OHCS return to control, while abundant C^{14} labeled polysaccharide remains in blood plasma(2). During the first few hours after injection, the bacterial polysaccharide progressively loses capacity to evoke a 17-OHCS response(2). Such observations suggest that a physicochemical change may have occurred in the polysaccharide after its administration. It has been demonstrated that the polysaccharide will not deplete adrenal ascorbic acid or decrease adrenal cholesterol in hypophysectomized rats(3). Further, Rosenfeld(4) was unable to increase *in vitro* production of adrenal steroids by perfusing isolated adrenal glands with different amounts of polysaccharides. Hypercorticism following administration of polysaccharide must, therefore, be due to increased secretion of ACTH by the anterior pituitary gland. The present investigation concerns *in vivo* and *in vitro* binding of C^{14} labeled polysaccharide with plasma proteins in relation to ACTH releasing capacity and the haptenic property of the polysaccharide.

Materials and methods. The preparation of C^{14} labeled (alkaline-extracted, haptenic, non-lethal) polysaccharide complex from *K. pneumoniae* has been described(5). The haptenic quality of isotopically labeled polysaccharide in circulating plasma was evaluated by quantitative precipitin technic using anti-*Klebsiella* rabbit plasma inactivated at 56°C. The original labeled polysaccharide was employed as a reference standard. The optimal range of the precipitin reaction was 1 to 5 γ/ml of rabbit plasma. Controls consisted of polysaccharide in 0.85% NaCl with normal rabbit plasma, polysaccharide in circulating guinea pig plasma with normal rabbit plasma and polysaccharide-containing plasma with 0.85% NaCl. Guinea pigs of either sex, weighing 200 to 250 g were given a single injection by ear vein with 20 γ C^{14} labeled bacterial polysaccharide/100 g body weight. The labeled polysaccharide was given in 0.15 M NaCl, 0.1 ml/100 g body weight, and a control group was given 0.1 ml 0.15 M NaCl/100 g body weight. Animals in both groups were killed ½, 1, 3, and 6 hours afterwards, employing cervical clamp technic(2). The heparinized plasma obtained by cardiac puncture was immediately centrifuged and plasma removed. Plasma 17-OHCS were determined by the method of Eik-Nes(6). Previously we isolated cortisol in guinea pig plasma(4) and plasma levels of 17-OHCS in the guinea pig apparently reflect the concentration of cortisol in this fluid. Following cervical clamp technic(2), plasma levels of 17-OHCS in normal guinea pigs will range from 1 to 30 γ/100 ml with mean concentration of 21 γ/100 ml

* This investigation was supported, in part, by research grants from Nat. Inst. of Health, U.S.P.H.S.
† Presented in part before Am. Soc. Exp. Path., Philadelphia, Apr., 1958.

TABLE I. Mean Plasma Levels of 17-OHCS (γ/100 ml) in Guinea Pigs.

Treatment	Hr after treatment ½	1	3	6
.1 ml of .15 M NaCl/100 g body wt	27 ± 10.0*(3)†	35 ± 10.0 (2)	31 ± 9.8 (3)	21 ± 3.7 (3)
20 γ polysaccharide/100 g body wt in .15 N NaCl	126 ± 14.0 (4)	175 ± 39.0 (5)	234 ± 21.0 (5)	101 ± 12.4 (3)
20 γ polysaccharide/100 g body wt (6 hr plasma *in vitro*)	175 ± 3.7 (2)	185 ± 22.0 (3)	115 ± 23.0 (3)	94 ± 11.0 (3)
20 γ polysaccharide/100 g body wt (12 hr plasma *in vitro*)	85 ± 9.0 (4)	179 ± 18.0 (3)	154 ± 6.9 (3)	50 ± 9.5 (3)
20 γ polysaccharide/100 g body wt (6 hr plasma *in vivo*)	99 ± 3.0 (2)	125 ± 9.0 (3)	59 ± 10.0 (2)	29 ± 8.0 (2)
20 γ polysaccharide/100 g body wt (12 hr plasma *in vivo*)	106 ± 10.0 (2)	120 ± 11.0 (2)	39 ± 9.0 (2)	28 ± 7.0 (2)

* Stand. error of mean. † No. of animals tested.

and standard error of this mean of ± 5.2. Normal animals were given C^{14} labeled polysaccharide intravenously and exsanguinated 6 or 12 hours later. The heparinized blood was immediately centrifuged and plasma removed. An aliquant of plasma was used for determination of radioactive carbon and the remainder of plasma was injected into recipient guinea pigs by the ear vein, each animal receiving 20 γ of C^{14} labeled material/100 g body weight in less than 0.2 ml plasma/100 g body weight. These animals were killed ½, 1, 3, and 6 hours afterwards and plasma 17-OHCS determined. These experiments will be referred to as: 6 or 12 hours *in vitro* plasma. Pooled, heparinized plasma obtained from normal guinea pigs exsanguinated by cervical clamp technic was incubated at 39°C for 6 and 12 hours with labeled polysaccharide. All incubations were done immediately after blood was withdrawn. Normal animals were given 20 γ of C^{14} labeled material in 0.33 ml incubated plasma/100 g body weight, and plasma 17-OHCS were determined in these animals ½, 1, 3, and 6 hours after injection of the incubated plasma. These experiments will be referred to as: 6 and 12 hours *in vivo* plasma. Paper electrophoresis of guinea pig plasma from *in vivo* and *in vitro* experiments, containing 100 γ and 60 γ of C^{14} labeled material, respectively, was carried out in a Durrum type cell with veronal buffer, pH 8.6, ionic strength 0.1, potential gradient 6 volts/cm for 16 hours. Using a strip counter, the isotope material was localized on the electropherograms. Proteins were detected by densitometric measurements after staining with bromphenol blue.

Results. Intravenous administration of 20 γ of C^{14} labeled polysaccharide/100 g body weight in 0.15 M NaCl (Table I) in normal guinea pigs resulted in elevation of plasma 17-OHCS statistically greater than that seen following injection of saline alone, handling of animals and cervical clamp technic (p 1 hr. $<0.02>0.01$; p 2 hr.<0.01; p 3 hr<0.01; and p 6 hr. <0.01). The incubation of the bacterial polysaccharide with guinea pig plasma reduced the 17-OHCS evoking capacity of the polysaccharide. After the 6 hour *in vitro* incubation, only the 3 hour plasma 17-OHCS concentration differed significantly from that produced by the same amount of polysaccharide in saline (p 3 hr.<0.01; p 6 hr. >0.50), whereas the 12 hour incubation seemed to reduce further the ability of the polysaccharide to maintain high plasma concentration of 17-OHCS in the guinea pig (p 3 hr. <0.01; p 6 hr. <0.05, >0.02).

Although the polysaccharide in circulating plasma retained its haptenic properties (Table II), the *in vivo* polysaccharide was less capable of maintaining a high plasma concentration of 17-OHCS than similar quantities of labeled polysaccharide given in 0.15 M NaCl (p 3 hr.<0.01; p 6 hr. <0.02, >0.01).

TABLE II. Quantitative Precipitin Test with Rabbit Anti-*Klebsiella* Plasma and C^{14} Labeled Haptenic Klebsiella Polysaccharide.

	Ratio of C^{14} polysaccharide to antibody/ml*	C^{14} in precipitate, %
Polysaccharide in .15 M NaCl + anti-*K*. plasma	37.5	74.7
	18.7	77.9
	9.7	84.1
	4.7	91.9
Polysaccharide in .15 M NaCl + normal rabbit plasma	38.8	1.8
	19.5	3.2
Polysaccharide in normal guinea pig plasma + anti-*K*. plasma	18.7	68.4
	19.5	73.8
Polysaccharide in circulating guinea pig plasma + normal rabbit plasma	39.0	1.8
	19.5	1.6
Polysaccharide in circulating plasma *(in vivo)* + anti-*K*. plasma		
6 hr after inj.	35.6	64.3
	17.8	78.9
	8.9	84.6
	4.5	93.8
12 hr after inj.	12.4	64.0
	6.2	92.6
	3.1	98.0

* Total volume of precipitin test, 0.4 ml. Ratio of hapten solution to anti-*Klebsiella* plasma, 1:1.

Electrophoretic studies disclosed that the bacterial polysaccharide did not migrate in veronal buffer at pH 8.6 (Fig. 1). After 6 or 12 hours incubation with plasma, the polysaccharide migrated with the slower moving plasma proteins but not with albumin (Fig. 1). The polysaccharide in circulating plasma 6 or 12 hours after injection tended to migrate in proportion to amount of plasma protein and was especially prominent at the albumin peak (Fig. 1). Relatively less was found in α, β and γ globulins at 12 hours than at 6 hours.

Comment. Six and 12 hours after injection of C^{14} labeled polysaccharide, 28 and 20% respectively of the original radioactivity was found in the circulating plasma. The data suggest that the remaining bacterial polysaccharide had combined with plasma proteins and thereby lost a considerable amount of its capacity to evoke a response in plasma 17-OHCS, although the haptenic property remained. The nature of such binding seems unspecific, since the C^{14} labeled polysaccharide migrated electrophoretically with α, β, and γ globulins after an *in vitro* incubation, while the polysaccharide subjected to plasma *in vivo* tended to become uniformly distributed among plasma proteins.

Hegemann(7) and Goodale and co-workers (8) found that the pyrogenic effect of polysaccharides was reduced by incubation with fresh human plasma. Rall, *et al.*(9) made similar observations with rabbit serum. Landy and co-workers(10) reported that polysaccharides from *S. marcescens, S. typhosa* mouse sarcoma 37, and human erythrocytes lost the capacity to produce hemorrhagic necrosis in mouse sarcoma after 1 hour incubation at 37°C with human and guinea pig sera. Mouse serum had only a slight effect and rabbit serum had none. Incubation of polysaccharides with serum also reduced the preparatory capacity of the polysaccharide for the local Shwartzmann reaction and for the skin-necrotizing effect of epinephrine(10).

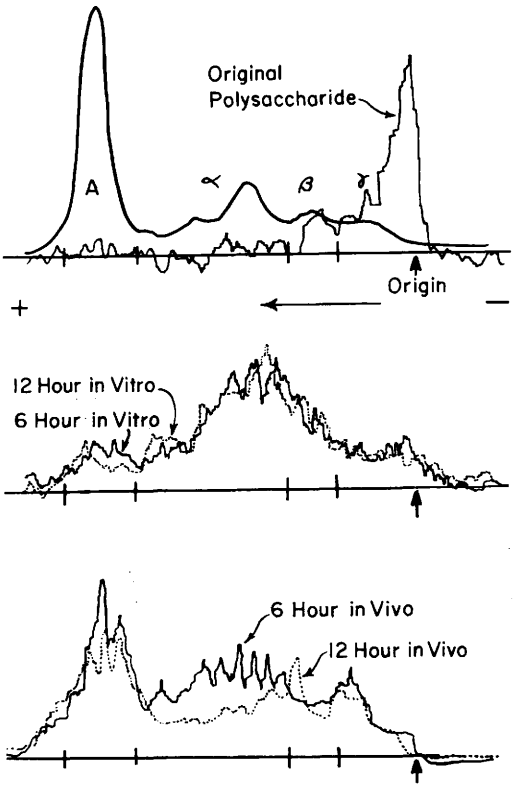

FIG. 1. Electrophoretic distribution of C^{14} labeled bacterial polysaccharide. A, albumin; α, α globulin; β, β globulin; γ, γ globulin.

Some attention has been drawn to the specific plasma protein components which may be effective in modifying various biologic responses of foreign polysaccharides. Landy, *et al.*(10) considered the properdin system to be unimportant in mouse sarcoma necrosis, while Cluff and Bennett(11) concluded that properdin associated with β globulin has an important role in inactivation of the pyrogenic effect with rabbit serum. Ho and Kass (12) found that Cohn's plasma fractions II + III, III and IV-1 reduced the lethal property of crude endotoxins in the rat. This may be related to modification of Ouchterlony precipitin bands with β globulin(13). Formation of polysaccharide-protein complexes is well known. Hyaluronic acid combines with various plasma proteins depending upon pH (14), heparin complexes with albumin(15), chondroitin sulfate binds with euglobulins (16), while bacterial polysaccharides, yeast zymosan and hog gastric mucin remove or inactivate properdin(17). Bernfeld *et al.*(18) reported that β-lipoglobulin and fibrogen form insoluble and soluble complexes with certain sulfated polysaccharides. These interactions with proteins involve relatively large quantities of polyanionic substances in contrast to our study with bacterial polysaccharide, in which the highest ratio by weight with protein is 1:300. Six and 12 hours after injection of the polysaccharide into the guinea pig, the ratio is approximately 1:1,000 and 1:1,500 respectively.

Apparently, increase in adrenocortical activity following polysaccharide administration is a result of ACTH release(3,4). ACTH may, therefore, be released from the hypophysis during the first moments after injection of polysaccharide and before protein-polysaccharide combination has occurred. Since the polysaccharide loses some of its ability to influence the pituitary-adrenal system, both by incubation with guinea pig plasma and during its circulation in the guinea pig, the combination between the polysaccharide and the plasma proteins may result either in blocking the part of the polysaccharide responsible for ACTH release or also making the polysaccharide less toxic and hence, less of a "stress" for the animal. The pathways through which the bacterial polysaccharide produces a stimulation of the pituitary-adrenal axis in the guinea pig are, however, not known.

Summary. A non-lethal, haptenic polysaccharide from *K. pneumoniae,* given intravenously to guinea pigs, produces a marked, but transient, elevation in plasma 17-hydroxycorticosteroids. Incubation of the polysaccharide with normal guinea pig plasma partially inactivates this response. The polysaccharide remaining in the circulating plasma 6 and 12 hours after intravenous injection shows a greater loss of this ACTH releasing property. Electrophoretic studies indicate that the polysaccharide is bound to plasma proteins, but there are marked differences in *in vitro* and *in vivo* binding with albumin. The bound polysaccharide, however, retains its haptenic qualities. These findings suggest that the bacterial polysaccharide undergoes little, if any, degradation but loses its 17-OHCS evoking capacity by combining with plasma proteins.

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Received June 10, 1958. P.S.E.B.M., 1959, v100.

Hypothermia: IV. Study of Hypothermia Induction Time with Various Pharmacological Agents. (24617)

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Hypothermia is of interest from its practical application(1) as well as from the observed basic physiologic alterations. At present, most of the interest in hypothermia concerns its application to cardiovascular surgery and neurosurgery(2). McQuiston(3) reported cases of 25 children subjected to various cardiac operations under hypothermia. Bigelow (4) and Cookson(5) performed dog experiments in which they occluded both vena cavae during hypothermia thus giving the surgeon a bloodless field for intra-cardiac surgery. Application of the principle of hypothermia for diminishing vital tissue oxygen requirements in patients undergong cardiac surgery was successful by Swan and his associates(6). Interest in hypothermia was aroused also by the concept of "artificial hibernation" developed by Laborit and Hugenard(7). They achieved their "artificial hibernation" through use of a "lytic cocktail" (a pharmacological mixture containing chlorpromazine, promethazine, and meperidine). Their major interest was to potentiate general anesthesia, and counteract the shock and other undesirable reactions to surgical or traumatic stress, but it was also noted that with supplementary body cooling, hypothermia could be obtained easily. Physiological alterations resulting from the lytic cocktail in patients undergoing surgery have been summarized by Shackman *et al.*(8). The effectiveness of the lytic cocktail for inducing hypothermia has been tested by Dundee *et al.* (9), who also concluded that muscle relaxants were equally effective. Moyer *et al.*(10) compared the effects of agents which block the sympathetic nervous system on hypothermia induction time. The rationale for this study was that the autonomic response to hypothermia supposedly provides a barrier to hypothermia induction. Adrenergic blocking agents (chlorpromazine and Hydergine)* which also possess central effects, Dibenzyline, an adrenergic blocking agent with minimal central effects, and mecamylamine (some central effect) were compared. They concluded that sympathetic nervous system depression *per se* did not affect hypothermia induction time and that chlorpromazine and mecamylamine (less effect than the former) were the only effective agents which reduced induction time due, apparently, to the anesthetic potentiating effect of these drugs. Our study was a further attempt to compare the effect of various pharmacologic agents on hypothermia induction time and to elucidate the locus of action of some of these agents. Chlorpromazine, the lytic cocktail containing chlorpromazine, promethazine, and meperidine, and d-tubocurarine were studied. Chlorpromazine and the lytic cocktail (promethazine, meperidine, and chlorpromazine) were compared to see how effective chlorpromazine plus adjunctive agents were, in contrast to chlorpromazine alone. Peripheral blockade of corticospinal pathways at the neuro-muscular junction by use of d-tubocurarine was studied to localize sites of action of effective agents. We were also interested in the effect of anesthesia alone compared to the more diverse pharmacological action of the various drugs.

* Equal mixtures of dihydroergocornine, dihydroergokyptine and dihydroergocrystine.