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Tissue Distribution of C^{14}-Labeled Bacterial Polysaccharide in Guinea Pig, Rat, Mouse and Rabbit.*† (24167)

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In the guinea pig the adrenal gland has the highest tissue concentration of C^{14} after intravenous injection of labeled *K. pneumoniae* polysaccharide - complex(1-3). C^{14} - labeled polysaccharide recovered by extraction of liver, spleen and adrenal partially retains the haptenic properties of the original polysaccharide(3). Apparently the bacterial polysaccharide(3), like C^{14}-labeled dextran in dogs (4), gradually disappears from animal tissues. Stark(5), however, studying mice injected with C^{14}-labeled pneumococcal polysaccharide, found the C^{14} content of splenic extracts almost unaltered for one year, while the antigenic property of such extracts was markedly reduced. The present study of distribution of *K. pneumoniae* polysaccharide in 4 common laboratory animals is pertinent to the species variation in physiologic responses and antibody formation following injection of bacterial products.

Materials and methods. The C^{14}-labeled polysaccharide-complex was extracted with 0.01 N KOH from *K. pneumoniae*, type B, which had grown at 37°C for 16 hours upon agar media containing 1-C^{14} acetate(3). The polysaccharide-complex had an isotopic activity of 0.678 μc/mg, was non-antigenic, but haptenic, and had no lethal toxicity. The polysaccharide was prepared as a 1% solution in 0.85% NaCl and sterilized by autoclaving. A single intravenous injection of the polysaccharide was given to guinea pigs (200 to 250 g body weight), Sprague-Dawley rats (100 to 130 g), Swiss albino mice (30 to 32 g) and New Zealand rabbits (2 to 2.5 kg). The rabbits received 0.5 mg/100 g body weight and the other species were given 1 mg/100 g body weight. Animals were sacrificed at 1, 3, 7, 14 and 30 days. Additional groups of guinea pigs and rats were given a single injection of the same quantity of polysaccharide by subcutaneous, intraperitoneal and intravenous routes and killed at 1, 3, 6, 12 and 24 hours and at 7 days. Determination of the C^{14} in plasma and tissue was carried out as previously described(3). C^{14} content is given as percent of injected dose and per gram dry tissue for liver, spleen and adrenals. The pooled mesenteric lymph nodes and pooled aliquots of liver of rats given a single intravenous injection of polysaccharide and killed at 7 days were ground in a Potter homogenizer and separated into insoluble and soluble portions by centrifugation. The polysaccharide fractions of lymph nodes and of liver were then removed from the soluble aqueous portion by the same technic used for extraction of the polysaccharide from *K. pneumoniae* and from liver, spleen and adrenal of the guinea pig(3). Precipitin tests were performed with heat-inactivated rabbit anti-Klebsiella serum and quantities of lymph node and liver extracts with C^{14} content equivalent to original bacterial polysaccharide/ml of antiserum in 1:1 dilution with 0.85% sodium chloride solution. C^{14} content of both precipitate and supernatant was determined(3). Controls

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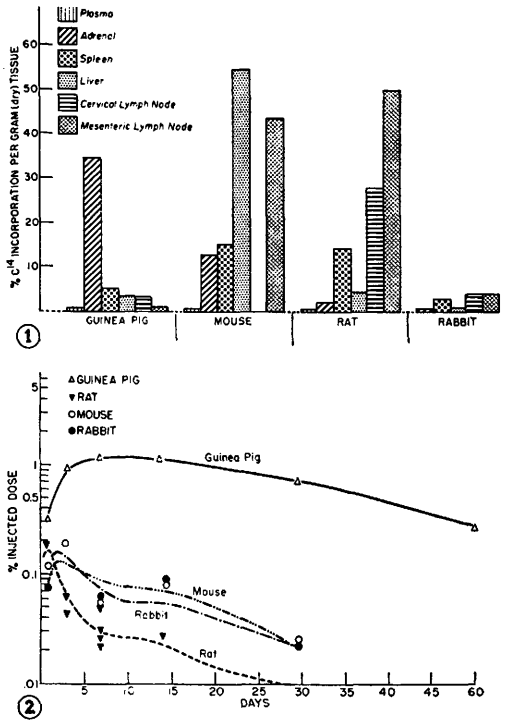


FIG. 1. % inj. C^{14} in 1 g (dry wt) of tissue 7 days after single intrav. inj.

FIG. 2. Comparison of C^{14} incorporation in adrenals of several animal species (intrav. inj.).

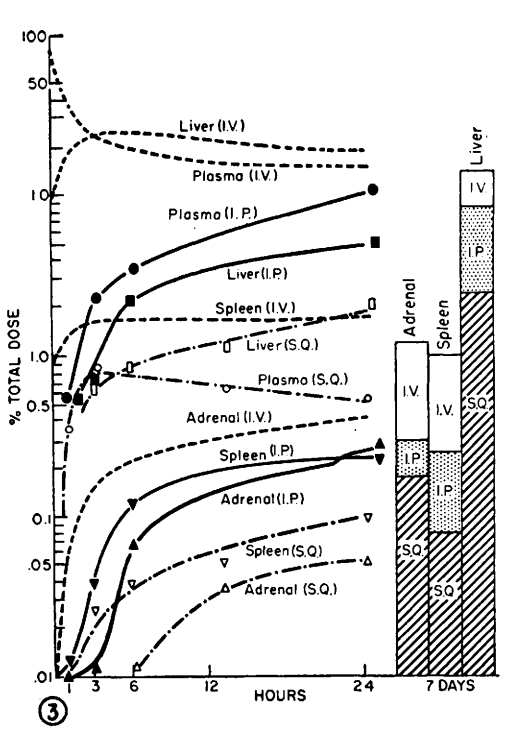
were a) comparable quantities of the original bacterial polysaccharide/ml of anti-Klebsiella rabbit serum and with normal rabbit serum diluted 1:1 with 0.85% sodium chloride and b) comparable quantities of tissue extracts with normal rabbit serum.

Results. Tissue distribution of C^{14} from intravenously injected, labeled bacterial polysaccharide varied markedly in different species (Fig. 1). Maximum C^{14} content/gram dry tissue was in the adrenal of guinea pig, liver and mesenteric lymph nodes of mouse, and mesenteric lymph nodes of rat (Fig. 1). C^{14} uptake by spleen was relatively higher in mouse and rat than in guinea pig and rabbit. Isotopic content of tissues of rabbit was lower than those of the 3 other species. Of the 4 species, only the guinea pig showed marked adrenal uptake of C^{14} (Fig. 1, 2). Negligible C^{14} is present in thymus, salivary glands, thyroid, ovary, testes, myocardium, skeletal muscle, brain, pancreas or intestinal wall of these animals. In blood, the isotopic label was almost exclusively in plasma and not in leukocytes or erythrocytes.

After its intraperitoneal injection, the C^{14}-labeled polysaccharide entered the blood stream more rapidly in the rat than in the guinea pig (Fig. 3, 4). Plasma levels of C^{14}-labeled polysaccharide in the rat were comparable to those following intravenous injection. However, after its subcutaneous injection, the polysaccharide appeared more rapidly in plasma of guinea pig than of the rat (Fig. 3, 4). During the first 24 hours after intravenous, intraperitoneal or subcutaneous injection of C^{14}-labeled bacterial polysaccharide, incorporation of label into liver, spleen and adrenal of both guinea pig and rat tended to reflect the plasma levels. Regardless of route of injected labeled polysaccharide, the mesenteric lymph nodes of the rat showed a higher incorporation of C^{14} than did the cervical nodes, while in the guinea pig the opposite was true.

The "polysaccharide" extracted from the mesenteric lymph nodes and liver of rats intravenously injected with labeled *K. pneumoniae* 7 days previously contained approximately 30% of the isotopic content of water soluble portion of tissue homogenate (Table I). The "polysaccharide" extracted from tissues had only a fraction of the specific activity of the original polysaccharide, but the labeled portion retained the haptenic quality (Table II).

Discussion. Variation in tissue distribution of different lipopolysaccharide endotoxins of Gram-negative bacilli may occur in the same as well as in different mammalian species. A considerable quantity of intravenously injected P^{32}-labeled *E. coli* endotoxin is taken up by liver and spleen of mice and guinea pigs(6). I^{131}-labeled somatic antigen of *Shigella flexneri* is also found in relatively high concentration in liver and spleen of the rat(7). However, Barnes, *et al.*(7), found little of the I^{131} somatic antigen in the cervical lymph node of the rat. Braude, *et al.*(8), reported a greater uptake of Cr^{51}-labeled *E. coli* endotoxin into liver of rabbit than observed in the present study after injection of C^{14}-*K. pneumoniae* polysaccharide. The marked



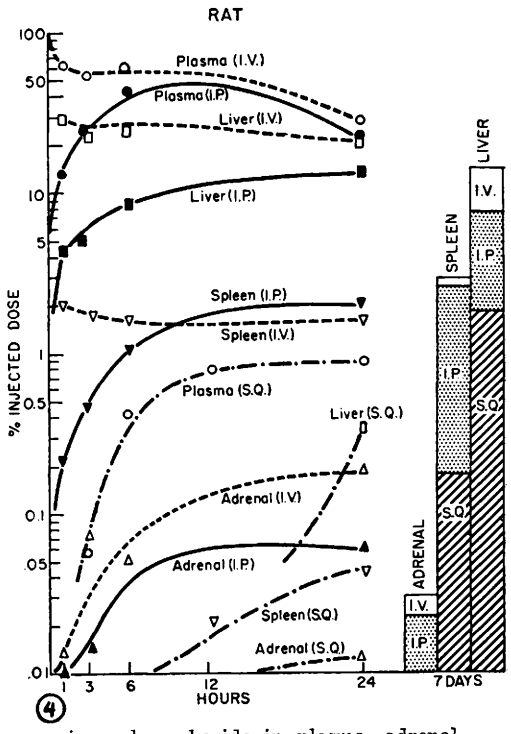


FIG. 3. Percent of C^{14} from labeled *K. pneumoniae* polysaccharide in plasma, adrenal, spleen and liver of the guinea pig after intrav., intraper. and subcut. inj.

FIG. 4. Percent of C^{14} from labeled *K. pneumoniae* polysaccharide in plasma, adrenal, spleen and liver of the rat after intrav., intraper. and subcut. inj.

uptake of label by the adrenal cortex after injection of C^{14}-labeled bacterial polysaccharide is found in the guinea pig, but not in rat, mouse or rabbit. In the guinea pig this adrenal uptake of C^{14} is reduced to the level of the other 3 species by pre-treatment with cortisol, but not by certain other steroids or ACTH(2).

It is of interest that C^{14}-labeled dextran has a distribution in dogs similar to that of *K. pneumoniae* polysaccharide in rodents. Terry, *et al.*(4), have found relatively high concentrations of C^{14}-labeled dextran in lymph nodes, liver, adrenal and spleen of dogs. Apparently the dextran is metabolized in dogs, for there is a decrease of labeled polysaccharide in tissues of the dog and a continued respiratory loss of $C^{14}O_2$. The metabolism of C^{14}-labeled dextran has also been studied in mice by Gray(9). Whole body homogenates of mice show an incorporation of C^{14} into protein and lipid, indicating the label had entered the general carbon pool.

Previous studies with C^{14}-labeled *K. pneumoniae* polysaccharide suggest that this bacterial product is metabolized by the guinea

TABLE I. Extraction of C^{14}-Labeled Polysaccharide from Mesenteric Lymph Node and Liver of Rat Injected with *K. pneumoniae* Polysaccharide and Days Previously.

				Tissue polysaccharide		
		Organ homogenate	Water-soluble fraction	Ethanol-insoluble, mg	C^{14} recovered, %	C^{14}/mg, % of bact. poly.
Lymph node	mg	328	137	16.9		
	mμc	171	147	45.0	26.3	.40
Liver	mg	3,280	1,900	50.4		
	mμc	100	64.8	33.0	33.0	.09

TABLE II. Quantitative Isotopic Precipitin Test with C^{14}-Labeled Polysaccharide Extracts from Mesenteric Lymph Node and Liver of the Rat.

	Original bact. poly.		Lymph node		Liver	
Rabbit serum	Quantity, μg/ml AB	C^{14} in precipitate, %	Quantity, μg/ml AB	C^{14} in precipitate, %	Quantity, μg/ml AB	C^{14} in precipitate, %
Normal	20.0	2.5	6.0	1.0	.14	1.9
	10.0	1.8				
	3.0	1.8				
Anti-Klebsiella	20.0	82.0	6.0	94.7	.14	78.6
	10.0	91.0	3.0	98.0		
	3.0	94.5				
	.1	90.0				

pig(3). $C^{14}O_2$ is lost in the respired air during the first 48 hours after intravenous injection of the polysaccharide, while urinary loss of C^{14} continues for as long as the tissues contain C^{14}. During the first week after injection, the C^{14} in plasma declines exponentially to very low levels and following this, remains almost free of label. This observation, plus the failure of adipose tissue to incorporate C^{14}, suggests that relatively little of the C^{14} from the labeled polysaccharide enters the general carbon pool during the metabolism of the *K. pneumoniae* polysaccharide. After injection of labeled *K. pneumoniae* polysaccharide, a considerable amount of C^{14} in tissues of guinea pig and rat retains the original haptenic property. By simple qualitative extraction of liver and spleen of the guinea pig(3) and lymph nodes and liver of the rat, labeled polysaccharide is recovered which possesses part or all of the original haptenicity. Additional qualitative evidence for persistence of bacterial polysaccharide in animal tissues has been provided by the fluorescent antibody procedure for *K. pneumoniae* capsular polysaccharide(10) and pneumococcal polysaccharide(11) and by tissue extraction and precipitin titers with non-labeled pneumococcal polysaccharides in the mouse(12).

Summary. Tissue distribution of C^{14}-labeled *K. pneumoniae* polysaccharide-complex has been studied in guinea pig and rat after intravenous, intraperitoneal and subcutaneous injection, and in the mouse and rabbit after intravenous injection. The polysaccharide entered the blood stream from the peritoneal cavity more rapidly in the rat than in the guinea pig, while absorption from subcutaneous tissues was more rapid in the guinea pig. After intravenous injection, there were marked differences in tissue concentration of labeled polysaccharide in the 4 animal species. By simple extraction procedures, approximately 30% of the label in liver and mesenteric lymph nodes of the rat has been recovered in a polysaccharide retaining the haptenic property of the original polysaccharide.

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