the principal tissues of the rat (excluding the thyroid) were also assayed for deiodinase activity. As in the case of the dog, only the liver and kidneys were found to possess activity.

Summary. It is believed that the salivary glands play no active role in metabolism of such thyroid analogues as DIT in the dog. The amount of iodide cleared into the saliva appears to be strictly a function of the circulating plasma level of iodide ion and is not influenced by exogenous thyroid hormone. This clearance will depend of course upon the rates with which exogenous diiodotyrosine, thyroxine and triiodothyronine become deiodinated. Since neither dog nor rat salivary glands appear to contain a DIT deiodinase system such as that found in liver and kidney tissue, it is concluded that these glands are not involved in the metabolism of DIT nor in the control of the circulating blood level of this analogue.

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Incorporation in Adrenal Cortex of C^{14} Labeled Fractions of *Klebsiella pneumoniae*.*† (21966)

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Crude polysaccharides from *Klebsiella pneumoniae* type B produce severe joint and mild cardiac valvular lesions in the guinea pig(1). To investigate the pathogenesis of the lesions, C^{14} labeled polysaccharides have been prepared and the distribution of the label followed in the animal tissues. The present report deals with (a) the adrenal cortical localization of the polysaccharide fractions; (b) comparison of incorporation of the polysaccharide with the whole organism and certain crude protein fractions in the adrenal; and (c) attempts to establish the mechanism.

Methods and materials. *Klebsiella pneumoniae* type B organisms were grown for 16 hours on peptone-glucose agar plates containing either 1-C^{14} sodium acetate or uniformly labeled d-glucose in concentrations ranging from 2 to 25 μc per ml of media. Bacteria were gently scraped from the agar. Crude polysaccharides were obtained by acid (A) and alkaline (B) extraction technics of Wong (3). The debris (C) remaining after acid extraction was separated into 2 fractions by cold trichloracetic acid to a concentration of 5% (w/v); the supernatant (D) was dialyzed against running water and evaporated to dryness over a steam bath. A portion of the trichloracetic acid precipitate (E) was washed with cold trichloracetic acid, then extracted 2 times with trichloracetic acid at 90°C for 10 minutes as for isolating nucleic acid (F)(4). Fractions (E) and (G) were insoluble in water and 0.85% sodium chloride and have not been studied in animals. The maximum absorption of the nucleic acid fraction (F) was at 260 mμ. Except for (D), the fractions were dissolved in 1% concentration in 0.85% sodium chloride, autoclaved and injected in less than 1 ml total volume into the ear veins of male and female guinea pigs

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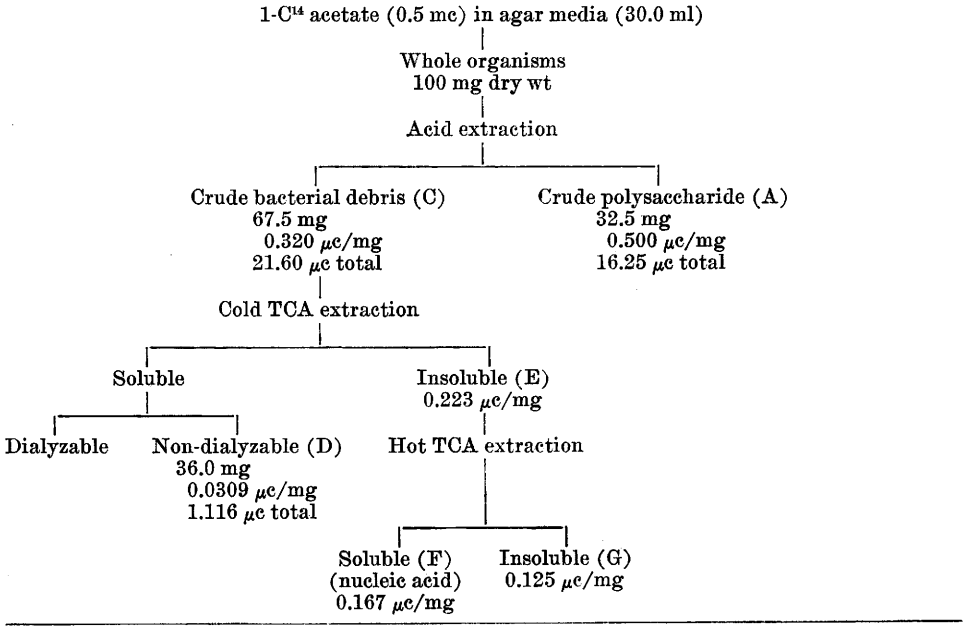
TABLE I. Incorporation of C^{14} into Certain Fractions of *Klebsiella pneumoniae*.



weighing 200-250 g at the beginning of the experiment. Whole bacteria were suspended in 0.85% saline, autoclaved and similarly injected. The lethal toxicity of the whole killed bacteria, and the bacterial debris (C), prevented the injection of quantities greater than 1.0 and 0.3 mg respectively. Urine was collected for the first 48 hours and at various daily intervals thereafter. During the collection of the urine, the guinea pigs were placed in glass containers and fed only cabbage supplement instead of their usual stock diet (Purina rabbit pellets). Animals were weighed weekly and sacrificed at varying intervals up to 2 months. Duplicate samples of organs or tissues were taken; one set was used for histologic examination and radioautography, the other set for grinding with distilled water in a Potter homogenizer, plating and counting in the proportional region with a windowless flow counter. The per cent incorporation in tissues or organs is expressed in two ways: (a) per total organ or tissue; (b) per g of dry tissue per kg body weight, ((activity per g tissue/ total dose) x (body weight) x 100). The latter affords a unit comparison of the activity in different organs and tissues, is useful for tissues of uncertain total weight such as lymph nodes, and compensates for the gain in organ weights with growth. Radioautographs were made by sandwich technics on no-screen x-ray emulsions with exposures up to 3 months at 4-6°C. This provides fair histologic detail, but unsatisfactory cytologic detail. For comparison with guinea pigs, 6 mice were injected with acid-extracted polysaccharide (A) by tail vein and sacrificed at 1, 3, 7, 14, 30, and 60 days and examined as above. As controls for the labeling of adrenal tissue with noncolloidal material, 1-C^{14} sodium acetate, d-glucose, d-sucrose, and dl-sodium glutamate 1-C^{14} were heat-sterilized and given alone and with 2 mg of labeled acid-extracted polysaccharide (A) in comparable isotopic amounts. To determine the chemical association of the incorporated crude polysaccharide in the adrenal cortex, the protein and lipid fractions of adrenal homogenates were separated by trichloracetic acid to 4% concentration (w/v) and ethyl ether extraction respectively. The radioactivity of the various fractions from these 2 procedures was determined.

As a control for reticuloendothelial activity, colloidal Au^{198} was injected into 3 animals sacrificed at 1, 3, and 7 days; tissue activities were determined and radioautographs were

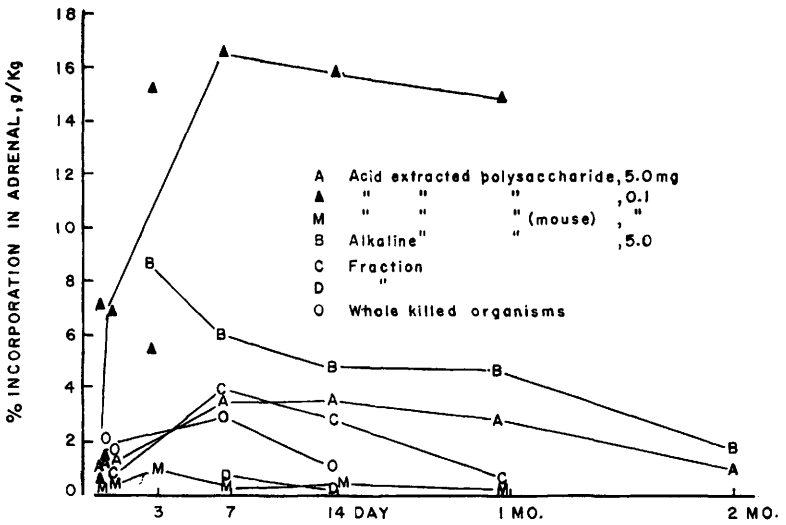


FIG. 1. Comparison of incorporation of various fractions of *Klebsiella pneumoniae* into the adrenal.

made in the same manner as for the other animals. Autoclaved undiluted Higgins india ink in ½ ml quantity was injected into 3 guinea pigs sacrificed at 3, 7, and 14 days; routine microsections were prepared.

Results. Incorporation into fractions of bacteria: The C^{14} of the acetate in the media is incorporated into all of the fractions of the bacteria which have been studied. The crude acid-extracted polysaccharide (A) has the highest activity and the cold TCA-soluble fraction (D), the lowest (Table I). The crude polysaccharide obtained by alkaline-extraction (B) has activity of 0.183 μc/mg in comparison with the crude bacterial debris activity of 0.3955 μc/mg. Extraction procedures have not been carried out on this bacterial residue. The polysaccharides obtained by the acid and alkaline extraction technics have a chemical composition similar to that previously reported(1).

Incorporation into organs and tissues of animals: After intravenous injection in guinea pigs of the various bacterial fractions, little remains in the plasma after 7 days (Table II). There is no appreciable labeling of the erythrocytes or leukocytes. The greatest loss in the urine occurs with the crude bacterial debris (C) and the nucleic acid (F). About the same percentage loss occurs with large as with small doses of the acid-extraction polysaccharide (A) (Table II). The activity in the urine parallels the activity in the plasma and is not in the form of carbonate or bicarbonate. The organs having the greatest activity, whether on total organ or unit-weight basis, are the adrenal, spleen, liver, lung, and cervical lymph nodes. The latter are consistently higher than the tracheobronchial or mesenteric lymph nodes; the mesenteric node is listed in Table II, for comparison with other studies. After the injection of the 2 crude polysaccharides, (A) and (B), the activity in the adrenal is well maintained for 4 weeks and is present in considerable concentration by the end of 2 months. By contrast, the activity in the spleen, liver, lung, and other organs declines after the first week and is barely detectable at the end of one month and not detectable at 2 months. While the greatest quantity is usually in the liver, the adrenal has by far the highest concentration per gram of tissue. The radioautographs reveal this activity to be in the zona fasciculata of the cortex and to be absent in the medulla (Fig. 2). In animals dying during the first day, the adrenals are engorged and microsections occasionally disclose zones of hemorrhage and minute areas of necrosis, usually in the inner cortex. In animals sacrificed at subsequent intervals, no histologic changes are detectable even though the radioactivity is relatively

TABLE II. % Incorporation in Guinea Pig Tissues at Optimal Time, 1 and 2 Weeks.

	I.V. dose, mg	Weeks	Plasma Total	Plasma g/kg	Adrenal Total	Adrenal g/kg	Spleen Total	Spleen g/kg	Liver Total	Liver g/kg	Lung Total	Lung g/kg	Mesenteric lymph node, g/kg	Pituitary, g/kg	Loss in urine, %
Acid extraction polysaccharide (A)	5.0	1	.14	.08	1.0	5.0	2.6	6.6	27.6	2.4	.6	.4	1.7		12
		2	.10	.05	1.2	6.1	1.4	3.5	11.9	1.0	1.1	.8	.3	.3	14
	.1	1	.09	.05	3.6	17.9	.8	2.0	18.9	1.6	.2	.1	.9	.7	7
		2	.00	.00	4.5	22.2	1.2	3.1	8.0	.7	.0	.0	.0		14
Whole, killed bacteria	.5	1	.11	.06	.8	3.9	.6	1.7	10.9	.9	.4	.3	.1	2.2	6
	1.0	2	.04	.02	.2	1.2	.4	1.1	6.0	.5	.1	.1	.1	.0	8
Alkaline extraction polysaccharide (B)	5.0	1	.17	.09	1.8	8.6	.4	1.1	2.5	2.1	.5	.3	.1	.0	14
		2	.03	.02	1.4	6.6	.3	.7	2.8	2.4	.2	.2	.2	.0	17
Bacterial debris (C)	.3	1	.25	.13	.8	4.1	.5	1.2	13.6	1.2	.2	.1	.1	.1	26
		2	.16	.09	.5	2.6	.3	.7	8.1	.7	.0	.0	.1	.0	73
Nucleic acid (F)	1.0	1	.03	.14	.0	.1	.0	.0	.0	.0	2.0	.1	.0	.0	31
		2	.00	.00	.0	.4	.0	.0	.0	.0	.4	.3	.0	.0	27
TCA soluble (D)	12.0	1	.07	.05	.1	.7	.4	1.0	4.9	.4	.1	.1	.1	.0	16
		2	.00	.00	.1	.6	.2	.4	3.5	.3	.0	.0	.2	.0	24

high. The simple extraction procedures with water, fat solvents, and trichloracetic acid show there is no significant labeling of lipid, but do not establish whether the labeled substances are protein or carbohydrate or both. Further investigation is, of course, needed to determine the nature of the labeled moieties. There is no appreciable adrenal incorporation of C^{14} from glutamic acid, glucose, sucrose, or acetate. In the spleen, the activity is in the red pulp, but not the Malpighian bodies and in the lymph nodes the radioactivity is present in the reticuloendothelial areas and not the lymph follicles. The distribution in mice is not the same as in guinea pigs, there being a greater activity in a gram of liver than of the adrenal or spleen.

India ink and colloidal gold: Variable sized particles of carbon from the india ink are found in the lung, liver, and spleen in the expected area. Some thromboses of pulmonary arteries and capillaries contain considerable black pigment. Fine black particles occur in the hepatic cord cells. Only traces of pigment can be detected in the microsections of adrenal. This pigment is in the sinusoidal epithelium and rarely in the cortical cells. The total activity and amount of colloidal gold given to the guinea pigs is not recorded, but the ratios of organ activities per gram dry weight at one week are as follows: Adrenal 1.0, spleen 23.36, liver 14.2, lung 0.13, mesenteric node 0.087, and pituitary 0.0157. Thus, colloidal gold does not have the distribution of the bacterial fractions and differs from the distribution of india ink particles in the lung and adrenal.

Discussion. The distribution of C^{14} labeled polysaccharides in the guinea pig is similar to that observed in mice and rabbits with I^{131} and dye-tagged antigen(5-7) and with fluorescent antibody(8) with the exception of a remarkably high concentration in the adrenal cortex of the guinea pig. In current theory there are three mechanisms for incorporation of the C^{14} label into the adrenal cortex: (a) cellular phagocytosis and storage of certain large molecular moieties of the nonhomogeneous bacterial fractions, (b) incorporation of a simple molecular constituent into a protein or carbohydrate component of

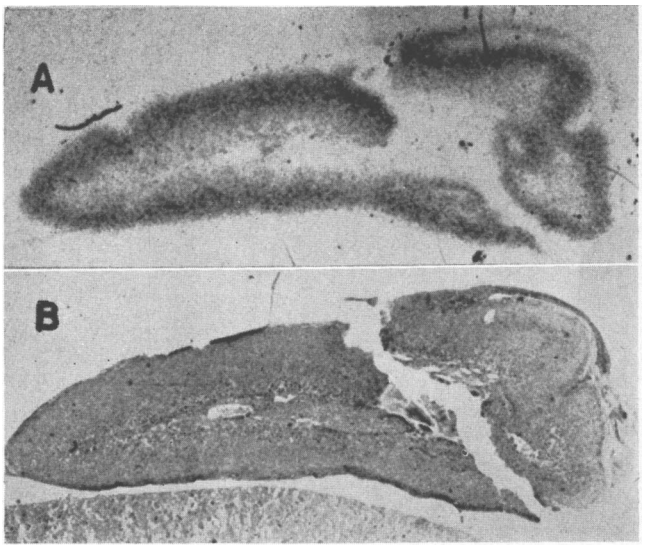


FIG. 2. Radioautograph of adrenal: (A.) of guinea pig given 5.0 mg of acid-extracted polysaccharide. Comparison with H & E stained microsection. (B.) reveals activity to be in outer cortex with slight activity in inner cortex and no activity in medulla. Exposure 6 wk on no-screen x-ray film.

the adrenal cortical cells, and (c) metabolic utilization of some simple molecular constituents such as a hexuronic acid.

The long persistence of the radioactivity in the adrenal argues against a metabolic utilization of a simple molecular constituent. The radioactivity also persists in the adrenal while disappearing from the accepted sites of more active reticuloendothelial activity such as spleen and liver. Studies on the behavior of colloidal substances do not clarify the appearance of the label in the adrenal cortex. Size alone has some bearing upon the localization in the different tissues of the reticuloendothelial system(9,10), particles of larger size showing rapid deposition in the liver and spleen and colloids of smaller particle size being deposited more slowly but in greater concentration in the bone marrow and spleen. In the former study(9), the adrenals contained only 0.002% of injected dose as compared to the bone marrow value of 44.0; spleen, 1.3; and liver 3.7%. Further study of the nature and behavior of colloids is needed to explain the differences in localization and storage of protein and polysaccharide antigens and of the various colloids of greater particle size. The values in mice as well as guinea pigs are quite different from the observations of antigen localization of Friedlander's capsular polysaccharide in mice(8) and of I^{131} protein antigens in rabbits(7).

The present study shows a rough parallelism between the degree of labeling of the bacterial fraction and the concentration of C^{14} in the adrenal. For example, the acid-extraction polysaccharide (A) comprises 32.5% by dry weight and 42.9% of the C^{14} in the bacteria, while the TCA-soluble fraction (B) of debris represents 36% by weight and 0.29% of the radioactivity. Depending on dosage given, the optimal polysaccharide label incorporation (% g/kg) in the adrenal is 6 to 22 while that of fraction (D) is but 0.7 (Table II). As yet a similar parallelism between reducing sugar content of the bacterial fraction and the concentration in the adrenal has not been established, but some chemical affinity of the adrenal cortex, whether for colloids or smaller molecules, is apparent.

The incorporation of such foreign bacterial products may prove to be of considerable significance in so-called stress responses, in the electrolyte disturbance and carbohydrate metabolism with infections, antigen antibody relationships, "collagen-vascular" diseases, and in ascorbic acid metabolism.

Summary. In the guinea pig the adrenal

cortex is the site of the greatest concentration of C^{14} after the intravenous injection of labeled polysaccharide and protein fractions of *Klebsiella pneumoniae*. This concentration persists for two months, while the activity of liver, spleen, lung and other organs declines and disappears. In general, the bacterial fraction with the highest incorporation of C^{14} from the media yields the greatest incorporation in the adrenal.

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Thioctic Acid Content and Pyruvate Oxidation in Ethionine-Damaged Rat Livers.* (21967)

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Recent investigations have established the role of alpha-thioctic acid (lipoic acid) (6,8 dithio-octanoic acid) as essential cofactor for metabolism of alpha-keto acids in both bacteria and animal tissue. This vitamin was first described by Kidder and Dewey as "Protogen," an essential growth factor for *Tetrahymena geleii*, present in liver and yeast; more recently its identity with "Pyruvate Oxidation Factor" of O'Kane and Gunsalus and "Acetate Replacing Factor" of Guirard has been established. The compound has been crystallized from liver and chemically synthesized. Thioctic acid is essential for oxidation of pyruvate by cells of *Streptococcus fecalis*, grown on thioctic acid-free medium, and is intimately associated with pyruvic oxidase and alpha-keto glutaric oxidase recovered from pigeon breast and pig heart muscle respectively. The role of this compound has been defined in the transfer to coenzyme A of the 2-carbon acetyl fragment obtained by oxidative decarboxylation of pyruvate and the 4-carbon succinyl fragment resulting from oxidative decarboxylation of alpha-keto glutarate. These developments have recently been extensively reviewed by Reed(15) and Gunsalus(7). The role of thioctic acid in animal nutrition has not been defined. No symptoms can be induced, nor can growth be diminished, in rats, by feeding a diet deficient in thioctic acid and simultaneously depressing possible intestinal bacterial synthesis of thioctic acid by feeding antibiotics(21). Feeding of an antimetabolite, the 8-methyl analogue of thioctic acid, which inhibits growth for some bacteria, produces no symptoms in chicks and rats(22). Impaired alpha-keto acid metabolism in severe human hepatic dysfunction (1, 2,19) and in experimental hepatic necrosis in rats(12,16) has been reported, and the importance of sulfur-containing amino acids in maintaining integrity of the liver is well recognized(5,16).

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