part of a different molecule. Unpublished observations, indeed, indicate that there is a difference in CA from *E. coli* O14 and from other bacteria, for CA from the former is largely insoluble and that from the latter soluble in 85% ethanol.

The present observations of the inhibitory effect of lipopolysaccharides, and probably of lipoid A as well, on the CA antibody response of the rabbit appear to explain the poor immunogenicity of CA present in the bacterial cells and in crude supernates from organisms other than *E. coli* O14. Moreover, eventual elucidation of the mode of action of lipopolysaccharides as inhibitors of the CA antibody response may throw light on basic mechanisms of antibody formation.

Summary. A common antigen (CA) is present in many species and serogroups of enteric bacteria, but only the antigen from *E. coli* O14 engenders CA antibodies in the rabbit upon intravenous injection. The ethanol soluble fraction of the former, however, induces the formation of CA antibodies. The present study has revealed that lipopolysaccharides (endotoxins) inhibit this CA antibody response. This inhibition was observed regularly when CA and lipopolysaccharides were injected as mixtures, but not when administered separately, although simultaneously, into different veins. Injection of mixtures prepared in ethanol were even less immunogenic than those prepared in water. A single preparation of lipoid A also inhibited the CA antibody response. It is postulated that the poor immunogenicity of enteric bacteria, other than *E. coli* O14, is due to the inhibitory effect of the lipopolysaccharide on the fully immunogenic CA.

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Salivary Gland Atrophy in Rat Induced by Liquid Diet.* (29699)

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The role of the nervous system in maintenance of structure and function of innervated organs is not completely understood. It is known, particularly from investigation of skeletal muscle(1) and salivary glands(2), that denervation markedly affects the morphological and physiological status of these organs, causing atrophy. It is not, however, clear to what extent these changes can be attributed to reduced activity of the organ as distinct from trophic influences unrelated to activity. The present data suggest that the innervation influences structure and function of the salivary gland largely by control of organ activity. It has been found that substitution of liquid ration for solid lab chow caused marked atrophy of the salivary glands of rat. Since these atrophic changes occurred when the innervation to the glands was in-

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tact, but when reflex stimulation of the gland was probably reduced, it is concluded that activity of the glands, rather than trophic influences of the innervation, plays a major role in maintenance of salivary gland structure and function.

Materials and methods. Three- to five-month-old rats of randomly-bred (Long-Evans) and inbred (Fischer, August, and Fischer × August hybrid) strains were maintained on either a standard laboratory pellet ration and tap water, or a liquid ration, given *ad libitum*. Liquid ration was prepared daily by mixing 2 parts water with one part of plain Metrecal powder, containing non-fat dry milk, soy flour, whole milk powder, sugar, starch, corn oil, coconut oil, dried yeast, chondrus extract, vit. A palmitate, calciferol, sodium ascorbate, thiamine hydrochloride, riboflavin, niacinamide, ferrous sulfate, potassium iodide, tocopheryl acetate, pyridoxine hydrochloride, calcium pantothenate, cupric carbonate, manganese sulfate, and imitation vanilla, or by mixing 5 parts of water with one part ground laboratory pellet ration. A Waring blendor was used to homogenize each ration. Both liquid rations were dispensed at room temperature and consumed, by licking, from a special container which automatically maintained the fluid level in a small (1.4 × 0.6 inches) stainless steel cup. After termination of the experimental period on liquid ration, rats were fasted for 24 hours (water available), and under secobarbital anesthesia (5 mg/100 g body weight), salivary glands were removed (bilaterally except in Long-Evans strain), weighed and portions were placed in Bouin's fixative for histological section, or immediately frozen for subsequent amylase determination. In experiments where saliva was collected, the secretions were obtained from cut ends of gland ducts following intraperitoneal injection of pilocarpine HCl (1 mg/100 g body weight)(3). Amylase was determined on appropriately diluted samples of saliva and gland homogenates by the method of Myers, Free and Rosinski(4), and expressed as mg of glucose formed per mg of gland or secretion, during a 15-minute digestion period at 37°C and pH 7.0. Na and K concentrations of salivas were determined with a Coleman flame photometer. Total protein determinations were made using Folin-Ciocalteau reagent(5), and protein was expressed as grams of crystalline bovine albumin. Electrophoretic separation of saliva proteins was made on paper in a Durram-type cell(6). Histological sections were stained with hematoxylin and eosin, and acinar cell counts were made from 25 fields in which only acinar cells were present.

Results. The 3 major salivary glands of rats maintained on liquid Metrecal ration exhibited marked atrophy (Table I), even though body weight, rate of body growth, and size of other organs (*e.g.,* liver, adrenal, kidney, etc.) were not impaired. Parotid showed the greatest decrease in gland weight (39-52%), with submaxillary and sublingual exhibiting smaller reductions (14-23%). Reduction in parotid weight occurred rapidly after instituting Metrecal ration, reaching nearly maximal values by the 4th day (Table II). The salivary glands of the rats maintained on liquid homogenized chow were reduced in weight to the same degree as those on Metrecal (Table I).

Histological examination of the salivary glands of rats fed Metrecal or liquid chow revealed a decrease in acinar-cell-size (atrophy) as the principal change. The acinar cells of parotid gland (the most markedly affected of the 3 glands) were reduced to about half the size of acinar cells in the control glands, as determined by direct measurement and as reflected by the nearly 2-fold increase in concentration of acinar cells in selected fields (Table II; Fig. 1). Generally, by 4 days these changes were nearly maximal. Ducts were not appreciably altered, although a tendency toward diminution of cell size of some ducts was observed. An increase in connective tissue was evident by 4 days.

A reduction in concentration of total proteins in the salivary secretion and in activity of amylase in the secretion and in the gland accompanied atrophy in the case of the parotid gland. On the basis of the change in activity, amylase concentration of the gland was reduced about 70%, and of the saliva about 40%; total proteins of the secretion were reduced 25% (Table III). Examination

TABLE I. Influence of Consistency of Ration on Salivary Gland Weight in Rat.

Strain of rat		Solid chow	Liquid Metrecal	% Decrease in gland wt	Liquid chow	% Decrease in gland wt	Metrecal; refed solid chow
August	PA	253 ± 9*	149 ± 6	41			
	SM	233 ± 8	196 ± 3	16			
	SL	36 ± 1	31 ± 1	14			
	Body wt†	247 (8)	281 (4)				
August × Fischer	PA	209 ± 6	118 ± 5	44	110 ± 5	47	
	SM	237 ± 9	191 ±10	19	186 ± 7	22	
	SL	43 ± 1	33 ± 1	23	37 ± 1	14	
	Body wt	246 (4)	263 (5)		224 (8)		
Fischer	PA	112 ± 3	69 ± 2	39			114 ± 3
	SM	120 ± 2	102 ± 2	15			119 ± 2
	SL	28 ± 1	24 ± 1	14			29 ± 1
	Body wt	150 (17)	156 (17)				150 (7)
Long-Evans	PA	256 ±23	122 ± 9	52			281 ±31
	Body wt	290 (7)	277 (10)				335 (4)

No. of rats shown in parentheses; animals maintained for 14 days on Metrecal or liquid chow; refed for 14 days.

* Mean gland wt, in mg, ± S.E., for parotid (PA), submaxillary (SM), and sublingual (SL).

† Mean, in g.

TABLE II. Relation Between Parotid Gland Weight and Length of Time on Liquid Ration in Rat.

Diet	Time on diet (days)	Body wt (g)	Parotid wt (mg)	% Decrease in gland wt	Acinar cell count (cells/unit area)
Solid chow	120	247 (8)	253 ± 9*		26.9 ± 1.0*
Metrecal	4	224 (5)	156 ±12	38	46.0 ± 1.2
"	14	252 (4)	145 ± 9	43	51.0 ± 1.4
"	28	281 (4)	149 ± 6	41	52.0 ± 1.1

No. of rats (August strain) shown in parentheses. * Mean ± S.E.

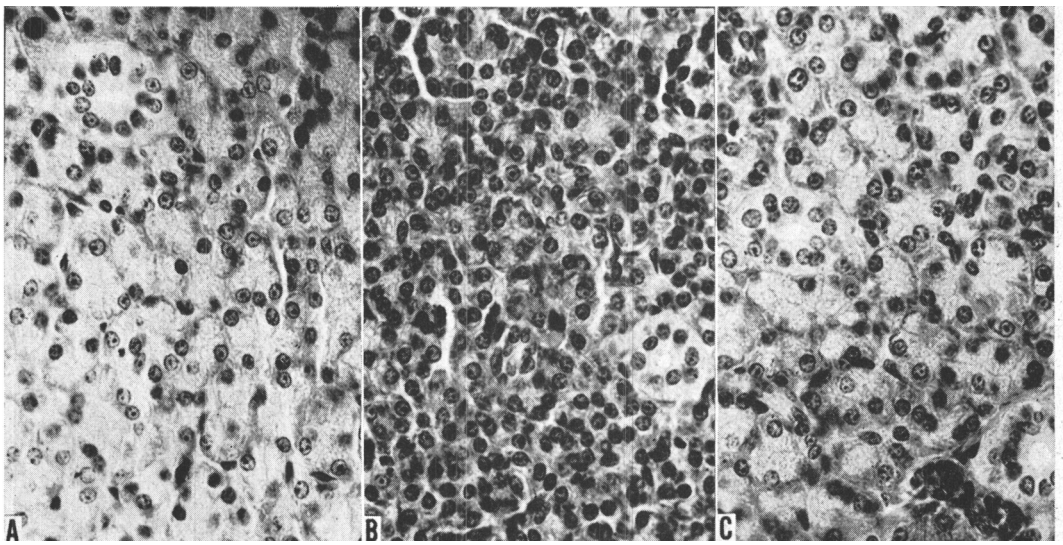

FIG. 1. (A) Normal parotid gland of August × Fischer rat fed solid chow ration; (B) parotid of rat fed Metrecal ration for 14 days; (C) parotid of rat fed Metrecal for 14 days, followed by 14 days of solid chow. Acinar cells show marked reduction in size (B) and return to normal size (C) (× 258).

TABLE III. Influence of Consistency of Ration on Protein and Electrolytes in Parotid Gland of Rat.

Diet	Gland amylase	Secretion			
		Amylase	Total protein	Na	K
Solid chow	497 ± 43*(6)	228 ± 15 (6)	4.10 ± .18 (9)	144 ± 3 (9)	18.7 ± 1 (9)
Metrecal†	138 ± 25 (10)	134 ± 9 (10)	3.33 ± .17 (4)	147 ± 7 (10)	21.5 ± 1 (10)
Metrecal; refed chow‡	425 ± 33 (5)	207 ± 15 (5)	4.39 ± .21 (5)	—	—

* Values in each case are means ± S.E.; No. of rats (Long-Evans strain) follow in parentheses.

Amylase is expressed as mg of glucose, formed during a 15 min digestion period at 37°, per mg of gland or ml of secretion; total protein is expressed as g %; Na and K are meq/l of saliva.

† Animals fed Metrecal for 14 days.

‡ Animals fed Metrecal ration for 14 days, followed by 14 days of solid chow.

of electrophoretic patterns of parotid secretion indicated that only some of the protein constituents of the saliva were altered. As shown in Fig. 2 and Table IV, components 4, 6, 9 and 12-13 were reduced in saliva from the atrophied gland, but the remaining 6 components were unaltered. There was no change in concentration of Na in the parotid saliva from atrophied glands and no appreciable change in K, although K tended to be slightly elevated (Table III).

The salivary glands of rats maintained on Metrecal showed complete return to normal structure (Table I, Fig. 1) and functional status (Tables III, IV, and Fig. 2) when the liquid ration was replaced by solid chow.

Discussion. The atrophic changes observed in the parotid glands of rats maintained on liquid ration are similar in kind and magnitude to those observed following postganglionic parasympathectomy of the parotid(2). In both cases, the gland is reduced in size, the acinar cells are smaller, and concentration of amylase in the secretion and the gland is reduced; Na and K concentrations of the secretion are very little altered. Preliminary experiments involving complete denervation of the parotid (removal of the superior cervical ganglion as well as severing of the postganglionic parasympathetic nerve) show results even more similar in magnitude to those observed when Metrecal is used as the ration.

The atrophy of the rat salivary glands induced by a diet of liquid Metrecal is attributed to the fluid consistency of the ration, and not to a specific factor in the Metrecal,

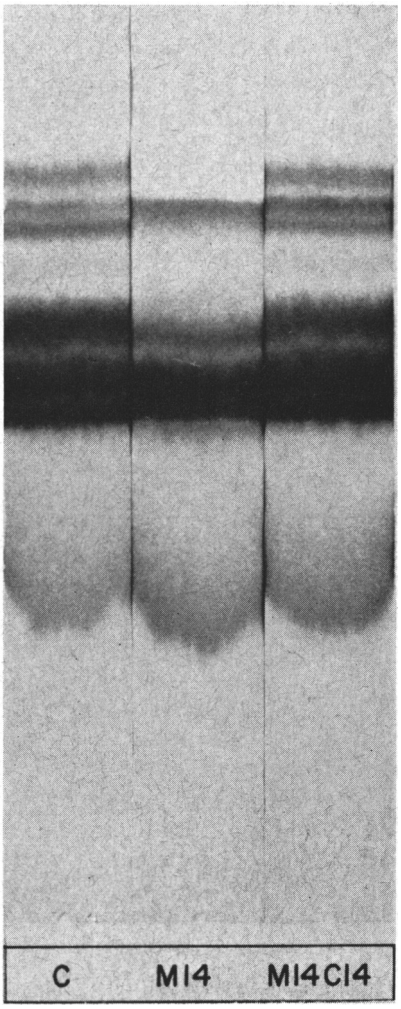

FIG. 2. Electrophoretic patterns of parotid salivas from rats fed solid chow ration (C), Metrecal ration for 14 days (M14), and Metrecal ration for 14 days followed by solid chow ration for 14 days (M14 C14).

TABLE IV. Influence of Consistency of Ration on Electrophoretic Components of Parotid Saliva.

Ration	Component (integration units) †										Total units
	1	3	4	5	6	7	8	9	10	12-13	
Metrecal‡ (4)	58 ±7*	10 ±1	36 ±3	76 ±4	31 ±1	10 ±1	7 ±1	9 ±1	15 ±1	1 ± .3	253 ± 9
Metrecal-chow§ (5)	57 ±5	9 ±1	73 ±5	82 ±4	54 ±4	7 ± .4	9 ±1	13 ±1	18 ±2	17 ±4	339 ±18
Chow (4)	51 ±8	7 ±1	81 ±5	81 ±2	65 ±3	8 ±1	10 ±2	16 ±1	12 ±2	17 ±6	348 ±18

No. of rats (Long-Evans strain) shown in parentheses.
* Mean ± S.E.
† Expressed as area beneath curve of Analytrol tracing.
‡ Animals fed Metrecal for 14 days.
§ Animals fed Metrecal ration for 14 days, followed by 14 days of solid chow.

or to nutritional deficiencies in general. Liquid chow caused similar atrophic changes, and no modification of total body weight from that of controls was observed with either liquid diet. Furthermore, the atrophic changes caused by feeding liquid Metrecal were fully reversed when a diet of solid chow was substituted for the Metrecal.

Rat parotid gland has not been observed to secrete "spontaneously"; stimulation by way of the innervation, or by administration of secretagogue drugs, is required to elicit any obvious secretion by the gland in the intact animal. Maintenance of intact animals on liquid diet reduces the extent of mastication and, hence, very probably decreases the degree to which the salivary glands are subjected to reflex stimulation. Therefore, the atrophy observed in the parotid gland of animals on liquid diet probably represents atrophy of disuse. While liquid ration obviates mastication, it would not necessarily eliminate gustatory, olfactory and other possible secretion stimuli. However, it appears that liquid chow and Metrecal may either be generally devoid of gustatory and olfactory qualities or that these may not be important secretory stimuli, since both rations produce similar degrees of atrophy.

Since these atrophic changes, similar to those observed following denervation, occurred in the face of an intact innervation, but when there is diminution of mastication and reflex stimulation of the gland, it appears likely that activity, dissociated from neurotrophic influences, plays a major role in maintenance of normal structure and function of these organs.

Summary. A diet of liquid ration (either Metrecal or liquid homogenized chow) caused atrophy of all major salivary glands in rats. No nutritional inadequacy accounted for the effects. The glands, as well as the acinar cells of these tissues, were markedly reduced in size; ducts were not appreciably altered. The parotid gland, the most affected of the 3, exhibited a decrease in amylase in the gland as well as in the secretion, but no appreciable change in concentration of Na and K in the secretion was noted. The functional and structural changes observed occur despite an intact innervation and suggest that activity of the glands, dissociated from neurotrophic influences, plays a major role in maintenance of gland integrity.

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