the amniotic cavity of chick embryos. It produced defects very similar to those previously observed with the highly nontoxic colloidal alumina. Carbon particles also produced striking defects of the same types as those produced by thalidomide and other noncolloidal but insoluble compounds which have been studied previously. Microscopic studies of tissues from chick embryos inoculated with carbon particles indicated that the particles entered the integumental ectoderm where they caused severe necrosis and sloughing of cells as well as marked localized hyperplasia. Following this the carbon entered the mesoderm, either through the denuded areas or, as observed in many instances, by passing through the ectoderm into the subjacent mesoderm. In the mesoderm the particles became located at progressively deeper levels, in some cases penetrating cartilagenous structures. Studies of the distribution of the carbon within the embryonic tissues indicated that defects occurred in structures developing in or near tissues in which the particles were located. Continual destruction of surface epithelium of the cephalic region which plays an important role in the development of skin, feather follicles, eyelids, nictitating membrane and cornea, resulted in defective development of these structures. When carbon was present in areas of the mesoderm from which bone or cartilage developed these structures were retarded or abnormal. Since the greater amount of carbon accumulated in the cephalic region (due to the tendency of carbon aggregates to gravitate toward this region) the frontal and squamosal bones and the sclerotic cartilages were chiefly affected.

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Metrecal-Induced Changes in Human Saliva.* (31781)

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Feeding a liquid diet of Metrecal to rats produces structural and functional changes of the salivary glands which are believed to represent atrophy of disuse(1,2). The liquid consistency of the diet results in reduced masticatory reflexes and the atrophy is related to consequent reduction in stimulation of the salivary glands rather than any nutritional factors. The structural and functional changes that result are particularly marked in the parotid and consist principally of acinar-cell atrophy and alteration in the water, protein, and electrolyte composition of the secretion. Furthermore, all changes may be completely reversed by reinstituting a diet of solid food. This evidence suggests that activity is an important determinant of the status of salivary glands in rats, but its role in maintenance of human salivary glands has not yet been determined. Consequently in the present investigations the effects of liquid diet on human salivary glands were examined. Since direct examination of the gland was not feasible, only the secretion from such glands was analyzed for changes indicative of atrophy. The results show that an exclusive diet of liquid Metrecal alters the secretion of water and

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protein from the parotid gland but not from the submaxillary gland. Furthermore, return to a regular diet restores secretory capacity of the parotid.

Materials and methods. Eight male dental students served as subjects for this study. Schneyer "segregators" (an apparatus for collection of separate submaxillary and sublingual salivary secretion from man(3)) were made for each subject with provision for collecting from the paired submaxillary glands only. In order to obtain a better seal for the segregators, 2-4 mm of acrylic were cut from the contacting surfaces and the voids replaced with rubber-base impression material. Each segregator was tested for leakage by placing phenolphthalein in the mouth and analyzing a test sample of saliva for the dye. Secretions were collected from each subject in the same quiet room at about 0800. Subjects ingested neither food nor water nor smoked during the 8 to 10 hours preceding collection. Each subject was seated in a comfortable chair and the pilocarpine (0.1 mg per kilo) was administered intramuscularly. A modified-Lashley cup and a Schneyer segregator were then placed in the mouth and the jaws held at rest. The first minute's flow was discarded and timed collections were begun into ice-cooled centrifuge tubes. Parotid saliva was collected for a period of 10 minutes and submaxillary saliva for 5 minutes. The saliva was refrigerated in a covered container for analysis later during the same day. Amylase activity was determined using the method of Myers, Free and Rosinski(4) and total protein determinations were made using the Lowry technique(5). A daily diet of 32 fluid ounces of vanilla Metrecal† (900 calories) was employed in these experiments. The Metrecal contained 7.4% protein, 2.1% fat and 11.6% carbohydrate and consisted of: concentrated sweet skim milk, milk protein concentrate, sugar, partially hydrogenated soy oil, artificial flavor, calcium cyclamate, chondrus extract, vitamin A palmitate, calciferol, sodium ascorbate, thiamine hydrochloride, riboflavin, niacinamide, ferrous sulfate, sodium iron pyrophosphate, sodium iodide, D-alpha-tocopheryl acetate, pyridoxine hydrochloride, cyanocobalamin, calcium pantothenate, cupric sulfate, and manganese sulfate. Prior to institution of the Metrecal diet, 2 determinations were made on each subject. In each case, the 2 values were similar and the mean of the 2 was used for the base-line value. Subsequently, single determinations were employed. Each subject then began a 900 calorie per day diet of liquid Metrecal. Water was the only other substance ingested. One week later, saliva was again collected and analyzed. Each subject was instructed to return to a regular diet for one week. Two subjects limited their regular diet to 900 calories/day; the caloric intake of the other 6 subjects was not restricted. At the end of the one-week period, saliva was collected and analyzed. Subsequent to the conclusion of the experiment, the 6 subjects who consumed an *ad lib* diet following one week of liquid Metrecal, were placed on a diet with restricted caloric intake of 900 calories per day. A week later, saliva was collected and analyzed. Body weight was recorded weekly throughout the experimental period.

Results. After a 7-day diet of liquid Metrecal, volume of parotid secretion was substantially reduced (Table I). The reduction occurred in every subject and varied in extent from 20-43% (mean of 34%). Reinstitution of a regular diet for seven days, however, increased volume of secretion to essentially base-line values. The difference between the post-Metrecal values and the corresponding base-line values was variable in direction of change as well as small in magnitude (—4% to +6%) suggesting that the differences represent experimental error. Similarly, total protein and amylase levels (with one exception) decreased, on the average, 30 and 29%, respectively, following a 7-day diet of Metrecal. A solid-food ration for one week also effected restoration of the ability of the parotid gland to secrete protein and water.

Submaxillary saliva, on the other hand, did not appear to be affected by a 7-day diet consisting only of Metrecal (Table II).

† Metrecal was kindly supplied by Dr. David J. Buddrus, Mead Johnson Research Center, Evansville, Ind.

TABLE I. Effect of Metrecal on Function of Parotid Gland in Humans.

	Subject	Pre-Metrecal solid food base-line (S_1)	Liquid Metrecal 7 days (M)	Post-Metrecal solid food 7 days (S_2)	% Change S_1—M/S_1	% Change S_1—S_2/S_1
Volume (ml/10 min)	DW	9.4	7.5	9.4	—20	0
	NS	18.6	13.2	18.3	—29	— 2
	TM	8.0	5.9	7.8	—26	— 3
	GG	6.3	4.0	6.2	—37	— 2
	HB	3.5	2.0	3.7	—43	+ 6
	BB	5.4	3.8	5.2	—30	— 4
	JS	6.7	3.8	6.8	—43	+ 1
	RG	5.3	3.7	5.5	—30	+ 4
		7.9*	5.5	7.9	—32	0
Total protein (mg %)†	DW	442	211	400	—52	—10
	NS	252	212	260	—16	+ 3
	TM	548	473	540	—14	— 1
	GG	481	270	495	—44	+ 3
	HB	488	342	500	—30	+ 2
	BB	428	340	400	—21	— 7
	JS	656	420	648	—36	— 1
	RG	436	332	450	—24	+ 3
		466	325	463	—30	— 1
Amylase (mg/mg)‡	DW	9.4	5.1	9.0	—46	— 4
	NS	21.7	21.8	21.6	0	0
	TM	20.0	16.2	19.6	—19	— 2
	GG	18.9	12.8	21.3	—32	+13
	HB	24.1	14.4	26.2	—40	+ 9
	BB	8.7	6.3	8.8	—28	+ 1
	JS	41.1	25.9	32.7	—37	—20
	RG	18.1	12.7	17.0	—30	— 6
		20.3	14.4	19.5	—29	— 1

* Mean.

† Expressed as mg of crystalline bovine albumin.

‡ Expressed as mg of glucose, formed during a 15 min digestion period at 37°, per mg of saliva.

Changes in the volume of secretion and total protein concentration were small (mean of —1% in both cases) and, in addition, variable in direction of change. Although amylase levels exhibited a somewhat greater change from base-line levels, the average change was again relatively small (mean of —9%) and variable in direction. For all 3 parameters of function investigated (water, amylase and total protein), the minor differences observed between base-line values and those obtained after reinstituting a diet of solid food are attributable to experimental error. Similar differences were observed after a 7-day diet of Metrecal, which strongly suggests that these differences also are not real. Thus it appears that the ability of the submaxillary gland to secrete water and protein is not affected by a diet of liquid Metrecal.

To rule out the possibility that the low caloric value (900 calories per day) of the Metrecal diet, rather than its fluid consistency, was responsible for the change in parotid gland function, the saliva from subjects who consumed solid food equivalent in caloric value to 900 calories per day was analyzed one week after instituting such a regimen. It is clear from the data in Table III that the restricted caloric regimen did not affect volume of secretion, total protein and amylase since these values did not differ from those obtained when the subjects were on a diet of solid food with caloric intake unrestricted. Furthermore, the weight loss following a diet of Metrecal (900 calories per day) or solid food (900 calories per day) averaged about 7 pounds per subject with each ration after one week. Thus, the changes in parotid gland function that occur following a Metrecal diet appear to be due to the fluid nature of Metrecal rather than to reduced caloric intake.

Discussion. Liquid diet in man causes

TABLE II. Effect of Metrecal on Function of Submaxillary Gland in Humans.

	Subject	Pre-Metrecal solid food base-line (S_1)	Liquid Metrecal 7 days (M)	Post-Metrecal solid food 7 days (S_2)	% Change S_1—M/S_1	% Change S_1—S_2/S_1
Volume (ml/5 min)	DW	17.7	17.8	17.6	+ 1	— 1
	NS	5.1	4.8	5.2	— 6	+ 2
	TM	7.1	7.1	7.2	0	+ 1
	GG	3.4	3.4	3.2	0	— 6
	HB	6.6	6.5	6.6	— 2	0
	BB	9.7	9.6	9.5	— 1	— 2
	JS	11.3	11.2	11.8	— 1	+ 4
	RG	10.5	10.4	10.8	— 1	+ 3
		8.9*	8.9	9.0	— 1	0
Total protein (mg %)†	DW	86	71	80	—17	— 7
	NS	110	125	115	+14	+ 5
	TM	149	159	155	+ 7	+ 4
	GG	85	80	90	— 6	+ 6
	HB	136	128	145	— 6	+ 7
	BB	134	135	130	+ 1	— 3
	JS	196	195	205	— 1	+ 5
	RG	142	143	140	+ 1	— 1
		130	130	133	— 1	+ 2
Amylase (mg/mg)‡	DW	0.4	0.1	0.4	—75	0
	NS	1.6	1.6	1.5	0	— 6
	TM	0.7	0.7	0.9	0	+29
	GG	0.5	0.4	0.5	—20	0
	HB	1.6	1.5	2.1	— 6	+31
	BB	0.4	0.6	0.6	+50	+50
	JS	1.2	1.0	1.9	—17	+58
	RG	0.9	0.9	1.1	0	+22
		0.9	0.9	1.1	— 9	+23

* Mean.
† Expressed as mg of crystalline bovine albumin.
‡ Expressed as mg of glucose, formed during a 15 min digestion period at 37°, per mg of saliva.

marked changes in the functional status of parotid gland, but the submaxillary, on the other hand, is unaffected. These changes consist in a marked reduction in volume of saliva and protein concentration of saliva. They do not appear to be due to nutritional deficiencies nor reduced caloric intake since the effects occur in the presence of a nutritionally adequate diet and are fully reversed by a low-calorie ration of solid food. With liquid diet, reduction in mastication (and therefore in the reflex-mediated secretory stimuli) occurs and activity of the parotid gland is thereby decreased. Other work also supports the view that chewing food stimulates the salivary glands in man(6). With liquid diet chewing is not required. In rats similar changes in protein and water secretion from the parotid gland occur with Metrecal or following reduction of gland activity by parasympathectomy(7) or total denervation(8). Although liquid Metrecal produces salivary gland atrophy in rat concurrently with these functional changes(1), it is not yet known whether human parotid and submaxillary glands atrophy under these conditions. The evidence does, however, suggest that activity plays a prominent role in regulating the functional status of parotid gland in man, although it does not affect the submaxillary gland.

The ability of liquid diet to alter the secretory capacity of the parotid gland (while submaxillary gland is unaffected) is not readily explained. One possibility is that the parotid is more susceptible to exogenous stimulation than the submaxillary gland. In man, when there is marked reduction in exogenous stimulation to the glands, the submaxillary secretion represents a far greater proportion of the total secretion than does the parotid(9). With increased exogenous stimulation, the proportion of the whole contributed by the parotid increases(10). In

TABLE III. Effect of Low Calorie Diet on Function of Parotid Gland in Humans.

	Subject	Post-Metrecal solid food 7 days *Ad lib*	900 cal/day	% Change
Volume (ml/10 min)	DW	9.4	9.5	+ 1
	NS	18.3	18.5	+ 1
	TM	7.8	7.9	+ 1
	GG	6.2	6.4	+ 3
	HB	3.7	3.6	— 3
	BB	5.2	5.6	+ 8
		8.4*	8.6	+ 2
Total protein (mg %)†	DW	400	470	+18
	NS	260	260	0
	TM	540	553	+ 2
	GG	495	483	— 2
	HB	500	488	— 2
	BB	400	435	+ 9
		433	448	+ 4
Amylase (mg/mg)‡	DW	9.0	9.9	+10
	NS	21.6	20.9	— 3
	TM	19.6	19.0	— 3
	GG	21.3	26.2	+23
	HB	26.2	27.2	+ 4
	BB	8.8	9.1	+ 3
		17.8	18.7	+ 6

* Mean.
† Expressed as mg of crystalline bovine albumin.
‡ Expressed as mg of glucose, formed during a 15 min digestion period at 37°, per mg of saliva.

the present study, where the exogenous stimulation is markedly increased by pilocarpine, the ratio of parotid and submaxillary saliva is about 50:50 (submaxillary saliva was collected from both glands simultaneously whereas parotid saliva was collected from one gland only) while in the "resting" state it is only about 30:70(9). It is also possible that the difference in response of the two glands to Metrecal is related to the differences in the role of the two branches of the innervation in maintaining gland activity and integrity. It has, for example, been shown that there are differential effects of denervation on gland structure and function, and these effects vary from one kind of gland to another(8,11).

The effect of a modest reduction in volume of secretion on the oral tissues of man is not known. More extensive reductions in flow, such as those which occur following large doses of X-irradiation to the salivary glands, result in rampant caries(12). In rat, extirpation(13) of the major salivary glands produces similar effects. Since reduction in volume (and composition) of secretion does occur with an exclusively liquid diet, it becomes apparent at least that inclusion of solid food in the diet may be generally important for maintenance of integrity of oral structures.

Summary. A marked reduction in volume and total protein and amylase content of the secretion from the parotid gland of man occurred when young adult men were maintained for 7 days on a diet consisting exclusively of liquid Metrecal. Restriction of caloric intake (900 calories per day) caused a reduction in body weight of approximately 7 lb per week when the diet was Metrecal or solid food; however, with the 900 calorie solid food diet, volume, amylase, or total protein of the secretion did not differ from those obtained from men maintained on an *ad lib* solid food diet. These changes in functional status of the parotid gland in man are attributed to a reduction in masticatory, and therefore, in the reflexly-mediated secretory stimuli to the gland. The submaxillary is not affected by the Metrecal treatment and a tentative explanation for these differences is presented.

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