2. Rabinowitz, J. C., Tabor, H., *J. Biol. Chem.*, 1958, v233, 252.

3. Grossowicz, N., Mandelbaum-Shavit, F., Davidcff, R., Aronovitch, J., *Blood*, 1962, v20, 609.

4. Chang, S. C., *J. Biol. Chem.*, 1953, v200, 827.

5. Donaldson, K. O., Keresztesy, J. C., *ibid.*, 1959, v234, 3235.

6. Herbert, V., Larrabee, A. R., Buchanan, J. M., *J. Clin. Invest.*, 1962, v41, 1134.

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Stability of Bacterial Flora with Long-Term Iodochlorhydroxyquin Therapy. (29088)

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A successful therapeutic and prophylactic control program, with the use of iodochlorhydroxyquin, for *Entamoeba histolytica* and *Shigella sonnei* dysenteries has been reported (1). The initial group of nearly 1300 patients has been expanded to approximately 4000 patients(2). Although long-term administration of iodochlorhydroxyquin has not resulted in any clinical manifestation of adverse bacteriological flora alteration, nor in any fungal disorder, it seemed advisable to confirm these clinical impressions with detailed bacteriological studies. Accordingly, a double-blind study was designed to investigate the possible bacterial and mycotic flora changes in the nasopharynx and intestinal tracts of subjects treated with iodochlorhydroxyquin.

Method. Twelve children, inmates of the Sonoma State Hospital, were chosen at random from the hospital population to be subjects for this study. Six were mongoloids (Down's Syndrome), and 6 had other diagnoses for the etiology of their mental retardation. All subjects were free from medical diseases other than the reason for hospitalization.

Drug and placebo therapy were administered in code by the trained nursing staff without any knowledge of what the medications were. Three mongols and 3 nonmongols, selected at random from the subject group, were given iodochylorhydroxyquin (Entero-Vioform®),* 250 mg three times daily with meals. The other 6 subjects (3 mongols and 3 nonmongols), also selected at random, were given placebo medication.

Specimens for bacteriological examination taken by swab from nasopharynx, throat, and rectum were submitted by code to an independent laboratory† not associated with the State Hospital. The microbiologist was unaware of which subjects received iodochlorhydroxyquin and which ones received placebo therapy. Control bacteriological examinations were performed before therapy on 2 occasions. Specimens were then taken at the end of 1, 2, 4, 8, and 12 weeks of continuous therapy.

Each nose, throat, and rectal swab was inserted into Stuart's transport medium and sent to the laboratory within 24 hours. Upon arrival, various types of culture media, which are widely employed for bacterial growth and identification, were inoculated from these swabs. Incubation was carried out overnight at 37° temperature.

After the incubation period, all growths were examined macro- and microscopically. Microorganisms present were subcultured by "pure" colony pick into broth. Confirmation of "purity" was established by Gram stain smear. If "pure," these subcultures served as inoculi for bacterial identification proce-

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TABLE I. Number of Subjects with Microorganisms in Areas of Culture Before and After 12 Weeks of Placebo Therapy.

Organisms	Control			During therapy		
	Nose	Throat	Rectum	Nose	Throat	Rectum
Hemolytic pyogenes var aureus	6	6	5	6	6	6
Strep viridans	6	6	0	4	5	5
Strep pyogenes	1	1	0	5	5	0
E. coli	0	0	6	0	1	6
A. aerogenes	0	0	0	0	0	0
P. aeruginosa	0	0	1	0	0	0
Anaerobes	0	0	0	0	0	0
Fungi	0	0	0	0	0	0

TABLE II. Number of Subjects with Microorganisms in Areas of Culture Before and After 12 Weeks of Iodochlorhydroxyquin Therapy.

Organisms	Control			During therapy		
	Nose	Throat	Rectum	Nose	Throat	Rectum
Hemolytic pyogenes var aureus	6	6	5	6	6	6
Strep viridans	4	5	0	0	5	0
Strep pyogenes	2	2	0	5	5	0
E. coli	0	0	5	0	1	6
A. aerogenes	0	0	1	0	0	0
P. aeruginosa	0	0	1	0	0	0
Anaerobes	0	0	0	0	0	0
Fungi	0	0	0	0	0	0

dures, sensitivity, resistance, and cross-resistance studies.

The sensitivity and possible development of resistance and cross-resistance was performed by the 2-fold serial tube dilution method. The media for iodochlorhydroxyquin was that of meat-extract, free phenol-red broth base; while for all other agents, the media was trypticase soy broth. The antibiotics used for sensitivity and resistance determination included chlortetracycline hydrochloride, tetracycline hydrochloride, oxytetracycline hydrochloride, erythromycin lactobionate, potassium penicillin G, dihydrostreptomycin sulfate, and matromycin.

Results. Tables I and II show the microorganisms found in the nose, throat, and rectal swab cultures prior to and after 12 weeks of iodochlorhydroxyquin or placebo therapy. Without exception, all aspects of the investigation were negative. There was no alteration of bacteriological flora in any of the cultured areas, regardless of duration of therapy. None of the isolated microorganisms demonstrated any change in the biochemical tests performed. No change in the sensitivity of any microorganisms occurred with iodochlorhydroxyquin or the various antibiotics utilized for the 2-fold serial-tube-dilution test. Cross-resistance to any of the antibiotics or iodochlorhydroxyquin was not observed. There was no isolation of a mycotic organism or anaerobes.

Discussion. The negative results obtained by microbiological examination of the flora from nose, throat, and intestinal tract following chronic administration of iodochlorhydroxyquin has answered two of the questions raised in clinical use of this medication. The bacteriological flora and antibiotic sensitivity of the "normal" constituents of the nasopharynx and intestinal tract are not altered with chronic administration of iodochlorhydroxyquin. The controlled study also confirms the negative findings of the hospital laboratory, where routine examinations of over 20,000 stool and rectal cultures from patients with long-term therapy revealed no alteration. Finally, none of the subjects in this study, and none of the 4000 patients who were treated with iodochlorhydroxyquin, have demonstrated or developed any stigmata of fungal disease.

Conclusion. 1. Long-term therapy with iodochlorhydroxyquin does not alter the "normal" microbiological flora of nose, throat, and intestinal tract. 2. Bacterial sensitivity of "normal" flora to iodochlorhydroxyquin and antibiotics is not decreased by long-term therapy with iodochlorhydroxyquin.

1. Gholz, L. M., Long-term prophylactic control of amebiasis and shigellosis. *Am. J. Trop. Med. & Hyg.*, 1962, v5, 452.

2. Gholz, L. M., Arons, W. L., Amebiasis and shigellosis: Single agent prophylaxis and therapy, 1963, submitted for publication.

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Calcium and Phosphorus in Human Parotid and Submaxillary Saliva.* (29089)

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Until recently the only available data on the calcium and phosphorus of human salivary secretions was derived from analysis of total calcium and inorganic phosphorus in "whole saliva," the mixture of secretions collected by expectoration(1). During the last few years there has been some study of the separately collected parotid secretions(2,3) and a preliminary report in which parotid and submaxillary saliva were compared(4).

Precise information as to the amount, type and properties of the calcium and phosphorus of the separately collected parotid and submaxillary secretions would be of considerable value in investigations of dental caries and dental calculus formation as well as in studies of salivary physiology. In the present study the parotid and submaxillary secretions from the same individuals were analyzed and compared as to content of total and nondialyzable calcium and phosphorus.

Material and methods. Parotid and submaxillary saliva samples were collected within a few minutes of each other from each of 28 adults aged 20-64 years. The parotid saliva samples were obtained with a modified Curby Cup and the submaxillary saliva with individually modified plastic collectors(5). Salivary flow was stimulated by application of 2% citric acid to the tongue 3 times per minute. Immediately after collection: a) a portion of each sample was analyzed for total calcium and inorganic phosphorus or stored for short periods at 4°C and then analyzed; b) another portion of each sample was placed in a Visking casing and dialyzed against 100 volumes of distilled water in the cold (4°C) for 48 hours. The contents of the bag were then analyzed for calcium, phosphorus and protein.

To determine the effect of storage at —20°C aliquots from some of the samples were stored in a deep freeze, thawed, homogenized in a tissue grinder to break up insoluble matter, and a portion dialyzed as above. The dialyzed and nondialyzed portions were analyzed as above. Several samples were collected and stored under mineral oil at —20°C, then thawed, homogenized and dialyzed. The dialyzed portions were analyzed for total calcium.

Initially, several methods for calcium were compared: EDTA-calcein(6), oxalate precipitation(7), and the Kerr modification of the glyoxal-bis (2-Hydroxyanil) reaction(8, 9). When ashed samples were used, all 3 methods gave comparable results. The glyoxal-bis reaction was preferable to the other two with respect to simplicity of technique and reproducibility of the assays. All cal-

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