reflex must outweigh the accelerator influence of the Bainbridge reflex which would also be elicited under these conditions.

If inspiration slows the heart by the consequences of reduction in intrathoracic pressure, the slowing should appear when the intrathoracic pressure is reduced by other means. Voluntary inspiration against resistance is one such means and the one which we have employed. However, when the intrapulmonary pressure is reduced to −20 mm Hg with the chest in the expiratory position, there is no slowing of the heart. Probably the Bainbridge reflex and the Schwiegk reflex, both of which are doubtless evoked, cancel each other's effect on the heart rate.

Thus we arrive at the conclusion that none of the cardioinhibitor reflexes discussed can explain the slowing under consideration. Some other factor must be postulated, such as the position of the chest itself or of the diaphragm as suggested by Sylla,[4] or some secondary consequence of the inspiratory position other than those already discussed.

In support of this conclusion may be cited the finding that the heart rate when the chest is in the inspiratory position with an intrapulmonary pressure of −20 mm Hg is somewhat slower than when the chest is in the expiratory position with the same intrapulmonary pressure. The fact that the slowing is not as great as when the intrapulmonary pressure is atmospheric may be attributed to the greater voluntary effort required to reduce the intrapulmonary pressure to −20 mm Hg when the chest is in the inspiratory position than when it is in the expiratory position. The cardiac acceleration accompanying the greater voluntary effort, it may be suggested, would increase the inhibitory irradiation from the respiratory center to the cardiac vagus center and so in part counteract the decelerating influence of the inspiratory chest position.

Summary and Conclusion. The slowing of the heart produced by sustained deep inspiration is not due to the reflex consequences of lowered intrathoracic pressure nor to any other hitherto recognized reflex mechanism. It is probably caused by some influence activated by the assumption of the inspiratory position of the chest.

[4] Sylla, A., *Klin. Woch.*, 1936, **15**, 849.

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Concentrations of Sulfadiazine in Human Saliva Attained by Chewing Paraffin Containing Sulfadiazine.

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It is now recognized that the sulfonamides are effective in the therapy of many severe infections of hemolytic streptococcic origin. The results with these compounds have been less satisfactory in the treatment of patients with streptococcic upper respiratory diseases. This is especially true of the milder types of infections such as acute pharyngitis and tonsillitis. In our own clinical experience, the ingestion of moderate doses of the sulfonamides by patients having acute pharyngitis and tonsillitis has occasionally been attended by disagreeable side effects from the drugs, which overshadowed the questionable therapeutic responses. We have attempted to treat such individuals by the local application to the pharynx of powdered sulfonamide preparations, or solutions and suspensions of the compounds. The results have been disappointing.

The possibility arose that more desirable therapeutic responses might be obtained if the tonsillar tissues and oral pharynx were subjected to high concentrations of a suitable

TABLE I.
Sulfadiazine Concentrations in Saliva and Blood of Individuals Chewing 1.5 g Paraffin Impregnated with 0.325 g Sulfadiazine.

Patient No.	Mg per 100 cc sulfadiazine in saliva 5 min	30 min	1 hr	2 hr	Mg per 100 cc sulfadiazine in blood 1 hr
1	338	307	308	400+	1.48
2	70.8	310	278.6	112.2	0.59
3	44.3	115.9	268	342	0.67
4	45.2	888	888	359	0.30
5	35.4	109	309	334	0.00
6	106	295	325	65	trace
7	72.2	354	56	32.4	0.88
8	366	590	228	91.3	1.18
9	20.6	103	285	320.0	0.88
10	32.4	400	216	29.5	trace
11	65	516	272	79.5	0.88
12	46.8	173	120	41	0.80

sulfonamide over a considerable period of time. Clinical and *in vitro* studies indicate that sulfadiazine possesses a high degree of antibacterial activity for Lancefield group A strains of hemolytic streptococci, which may cause acute tonsillitis, acute pharyngitis, as well as scarlet fever. An approach to this problem was evolved by mixing sulfadiazine with melted paraffin, and having human subjects chew on the hardened paraffin. In this manner, remarkably high concentrations of sulfadiazine were maintained in the saliva. At this time, we are presenting a report of these observations.

Paraffin blocks, suitable in size for chewing, were impregnated with crystalline sulfadiazine in the following manner: 4.2 g (60 grains) of sulfadiazine was thoroughly mixed with 15 g of melted paraffin. The paraffin was then poured into a mold such as used for making twelve standard-sized rectal suppositories. Under these conditions, each "suppository" contained 0.375 g sulfadiazine, or slightly more than 5 grains. Human subjects were instructed to chew the paraffin, and samples of saliva were collected at stated intervals for the determination of sulfadiazine (unconjugated) concentrations. The determinations were made according to the method of Bratton and Marshall.[1]

The results with one group of 12 subjects are presented in Table I. Samples of saliva for sulfadiazine determinations were collected at the end of 5 minutes and 30 minutes, and after one and 2 hours. A specimen of venous blood was obtained after the paraffin had been chewed for one hour.

A second group of 8 patients was given a gelatin capsule containing 0.325 g of sulfadiazine. Samples of saliva were collected at the same intervals as in the preceding group, and samples of venous blood were obtained after the paraffin had been chewed for one, 2, and 3 hours. The sulfadiazine concentrations are shown in Table II.

TABLE II.
Sulfadiazine Concentrations in Blood of Individuals Receiving by Mouth One Gelatin Capsule Containing 0.325 g of Sulfadiazine.
Under same treatment there was no sulfadiazine in 100 cc saliva when tested 5, 30 min, 1, 2 hr.

Patient No.	Mg per 100 cc sulfadiazine in blood 1 hr	3 hr	6 hr
13	1.03	2.06	2.06
14	1.04	1.40	1.92
15	0.60	0.80	1.03
16	0.60	0.90	2.20
17	1.20	1.48	1.48
18	trace	1.48	2.36
19	1.48	2.06	1.62
20	1.20	1.48	2.10

It is of interest that in individuals receiving full therapeutic doses of sulfadiazine so that blood concentrations of sulfadiazine were maintained between 5 and 10 mg per 100 cc, the levels of sulfadiazine in the saliva ranged between 3 and 4 mg per 100 cc.

A study of the therapeutic results with the paraffin-sulfadiazine preparation in patients with acute pharyngitis or tonsillitis of streptococcic origin has not progressed far enough

[1] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

to warrant any conclusions at this time. This investigation will include cultures of material from the oral pharynx before, during, and after treatment with paraffin-sulfadiazine. The observations made to date are given so that other groups may carry out investigations relative to the therapeutic value.

Summary. When 0.325 g of sulfadiazine is incorporated into approximately 1.5 g of paraffin, and the paraffin-sulfadiazine mixture is chewed by human subjects, high concentrations of free sulfadiazine were attained in the saliva. Such a procedure may favorably affect the course of acute hemolytic streptococcic infections such as pharyngitis and tonsillitis without recourse to larger systemic doses.

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Immunologic Studies in Experimental *Trypanosoma cruzi* Infections: 2. Slide Agglutination and Intradermal Tests.*

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Whereas the antigenic composition of bacteria is fairly well known, little work has as yet been done on the antigenic composition of protozoa pathogenic to man. Flagellar 'H' and somatic 'O' agglutinogens have been demonstrated by the writer[1] for *Leishmania tropica,* and positive skin tests have been obtained by the writer[1] with the polysaccharide and protein fractions of *L. tropica* in cutaneous leishmaniasis.

The Machado[2] complement fixation test, using as antigen glycerine and aqueous extracts of the heart and spleen of infected puppies, has been used for the diagnosis of "Chagas" disease in man, and more recently[3] cultures of *T. cruzi* have been utilized as antigen. Precipitin[4] and agglutination[5] tests have also been used in experimental *T. cruzi* infections. Rabbits were immunized with killed and living cultures of *T. cruzi,* and their agglutinin titers ranged from 2,000 to 130,000, with an average of 2,000 to 4,000. Intravenous method of immunization gave rise to higher titers. Rabbits and guinea pigs which were given living cultures showed a lower titer (256 to 1,024). Sera of animals infected with *T. brucei* or *T. hippicum* agglutinated *T. cruzi* in very low titers (1:32). Lysins[6] for *T. cruzi* have also been observed.

Preparation of antigens for immunization. Five strains of *T. cruzi,* 2 arthropod strains from Texas (Packchanian, Davis), one armadillo strain from Brazil (Tatu) and 2 human strains, one from Brazil (1114) and another from Panama, were each cultured on modified Senekjie's medium[7,8] in Roux bottles or Erlenmeyer flasks for 7 to 10 days. The growths were emulsified in physiologic salt solution and killed in 0.3 % formalin. After 24 hours the trypanosomes were collected by centrifugation, and were suspended in 0.5 % phenol in saline. The suspension was standardized to contain 30 million trypanosomes per cc (by making counts in the blood-counting chamber).

Rabbits were immunized with respective cultures by intravenous injections. The dosage

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