greater concentration, but a smaller total amount than the red cells. Studies are now being conducted to determine the optimum concentration of thiouracil in the thyroid and the time required to attain this level.

The present report is a preliminary one dealing with the early response of thyrotoxic patients to treatment with thiouracil. The assessment of the full value of this drug must await prolonged and extensive studies, including careful observations for signs of toxicity.

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EFFECT OF VARIOUS CHEMICAL AGENTS AFFECTING PERMEABILITY OF THE MUCOSA ON THE FORMATION OF ULCERS

SCHIFFRIN[1] produced ulcers in the cat by running a 3 per cent. solution of pepsin in 0.1 N hydrochloric acid through a segment of the jejunum, but was unable to bring about ulceration when the acid alone was used. He emphasized the important role of pepsin in the formation of ulcers and was able to prevent the lesions by protecting the mucosa with colloidal aluminim hydroxide and aluminim phosphate.

The purpose of the investigation reported here was to study the influence of certain substances known to have marked effects in lowering surface tension or in otherwise increasing permeability of the mucosa to insulin, on the incidence and extent of the ulcerations initiated by pepsin and HCl. Chemically pure pepsin and insulin have similar molecular weights (unit weights by the ultra-centrifuge).

The method used in these studies was as follows. An incision was made in a dog under dial-urethane anesthesia, and the jejunum was brought to the surface of the abdomen. Three or 4 segments about 3 inches long, chosen in such a manner as to insure a good blood supply to each loop, were cannulated and reinserted into the abdomen, which was then closed. Solutions, preheated to 37° C., were allowed to flow by gravity through the loops at the rate of 3 cc per minute for 12 hours, or until a perforation occurred in one of the loops. The loops were then removed, split along the mesenteric line and examined grossly and microscopically. Thirty-two dogs were used. The particular loops used were not examined for spontaneous ulcers before perfusion, because to do so would require sacrifice of them before the experiment. However, in a large number of normal dogs used in this and other investigations[2] the spontaneous occurrence of a discrete ulcer has never been seen.

The concentrations of the substances used were: 0.1 N hydrochloric acid, 2 per cent. powdered U.S.P. Merck pepsin, 0.03 per cent. calgon (sodium hexametaphosphate), 0.1 per cent. hexylresorcinol, 0.05 per cent. pinacol (tetramethyl glycol), and 1 cc of methyl salicylate per liter of solution.

RESULTS

Table 1 gives the number of times a given solution was tried, the number of times ulcers were formed, and the percentage of this occurrence with the various solutions. Also included are the pH's of the solutions.

TABLE 1

OCCURRENCE OF ULCERS IN PERFUSED LOOPS

Solution	pH	Number of loops used	Number of times loops used appeared with ulcers	Percentage occurrence of ulcers
2 per cent. pepsin in distilled water	3.80	3	0	0
0.1N HCl	0.90	4	0	0
0.1N HCl and 0.1 per cent. hexylresorcinol.	1.05	3	0	0
0.1N HCl and 0.1 per cent. methyl salicylate	1.00	3	0	0
0.1N HCl and 0.03 per cent. calgon	1.15	6	1	16.6
0.1N HCl and 0.05 per cent. pinacol	1.00	3	1	33.3
0.1N HCl and 2 per cent. pepsin	1.10	22	6	27.3
0.1N HCl and 2 per cent. pepsin and 0.1 per cent. methyl salicylate	1.00	8	4	50.0
0.1N HCl and 2 per cent. pepsin and 0.1 per cent. hexylresorcinol.	1.10	13	7	53.0
0.1N HCl and 2 per cent. pepsin and 0.03 per cent. calgon	1.10	13	9	69.2
0.1N HCl and 2 per cent. pepsin and 0.05 per cent pinacol	1.10	7	5	71.4

In the loops through which pepsin alone was run the tissue appeared to be perfectly normal. When hydrochloric acid was run alone, the villi were sometimes partly destroyed. Hydrochloric acid with pepsin usually caused the villi to be destroyed with the frequent appearance of small ulcers. Hydrochloric acid with hexylresorcinol, calgon, pinacol or methyl salicylate usually produced some destruction of the villi. When calgon, pinacol, hexylresorcinol or methyl salicylate were introduced with both hydrochloric acid and pepsin, the submucosa was completely destroyed, usually showing black areas, as well as a high percentage of ulcers. On numerous occasions as many as 10 to 15 ulcers would appear in one loop when either of these compounds was used with HCl and pepsin. This was never the case when they were not used.

[1] M. J. Schiffrin, *Proc. Soc. Exp. Biol. and Med.*, 45: 592, 1940.

[2] R. L. Driver and J. R. Murlin, *Am. Jour. Phys.*, 132: 281, 1941.

DISCUSSION

For a long time it has been a mystery to physiologists why digestive juices of the stomach ordinarily do not digest the tissue which goes to form the alimentary canal while ingested tissue is readily digested. Driver and Murlin[2] showed that certain substances can increase the penetrability of intestinal mucosa by a protein molecule, insulin, as judged by its effect on blood sugar. Of these compounds, all that have been tried, namely, calgon, hexylresorcinol, pinacol and methyl salicylate, brought about a tremendous increase in the number and severity of ulcers.

Calgon is a compound noted for its ability to tie up calcium ions.[3] Since this ion has been reported to inhibit absorption[4,5] and since calgon was found to promote the absorption of insulin,[2] it seemed reasonable to expect an increased penetrability by pepsin with a consequent incidence of ulcers when calcium was rendered non-ionic and therefore non-absorbable in the intestine. Calcium combines with lipids and proteins to produce a hardening and protective effect on the mucosa. It is not inconceivable that the beneficial effects of milk in the treatment of ulcers may be attributed in part to its calcium content, and it would seem that fortification of milk with a calcium salt is indicated.

Hexylresorcinol is a powerful surface tension lowering agent and produces structural changes in the membranes of the mucosa which modify permeability.[2,6] Methyl salicylate exerts a special irritating influence on mucous membranes which increases permeation of protein molecules. Pinacol has little irritating effect and does not lower surface tension appreciably. This compound probably changes the penetrability of the mucosa by proteins by its particular molecular configuration, the hydrophobic and hydrophilic groups acting as a lock and key mechanism. The formation of ulcers in these experiments was not due to a difference in pH of the solutions. We conclude that substances which increase the permeability of intestinal mucosa by a lowering of surface tension or other means facilitate the formation of ulcers.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ON THE STAINING OF YEAST SPORES

ALTHOUGH it may be unnecessary to resort to staining procedures to demonstrate spores in some cultures of yeasts, frequently such stains are desirable for proof of the presence of spores or the proper delineation of these. As no one method of staining is used universally, each investigator uses the method of his choice, and this often is one of the methods used for bacterial spores. Thus, Henrici,[1] in a comprehensive review of the cytology and taxonomy of the yeasts, recommends the familiar technique of Moeller. This consists of primary staining with steaming carbol fuchsin, and, after proper destaining, counterstaining with Loeffler's methylene blue.

The above method, as used by the author, often gives slides with considerable precipitate, and frequently color differentiation between spore and vegetative cell is not clear. It seemed possible that the malachite green technique of Schaeffer and Fulton[2] might be more satisfactory since one step, the destaining procedure, is unnecessary. In this technique washing with water is sufficient to remove the primary stain from the vegetative cell. Only a few trials were needed to verify this assumption. Exceptionally clear differentiation was the usual result. Review of the literature revealed that Shimwell[3] had tried the technique with yeasts, although he modified the procedure by using alcohol as a destaining agent. Our results have not indicated the need for this.

The use of 5 per cent. malachite green as suggested by Schaeffer and Fulton for bacterial spores proves somewhat expensive if large numbers of slides are to be stained, as for example, in student laboratory exercises. It is possible to lower the concentration of the dye, and thus reduce the cost of the stain. This and other modifications have produced a staining technique with which we have had uniformly good results with spores of various yeasts. The brilliant contrast between the color of the spores and vegetative cells permits rapid identification, and is so striking that the novice experiences no difficulty in interpretation. In this laboratory, it has been much more successful than the technique of Gray[4] which employs a mixture of malachite green and basic fuchsin.

A number of modifications of the details of the Schaeffer and Fulton formula and procedure have been tested and the formula given below seems to be

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[5] H. Drawert, *Ber. Deutsch. Bot. Ges.*, 55: 380, 1937.
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[3] J. L. Shimwell, *Jour. Inst. Brewing*, 44: 474, 1938.
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