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A Technic for Separation of the Cells of the Gastric Mucosa.* (28086)

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Cellular biochemical and metabolic activity is most suitably investigated in a preparation of a single cell type. A suspension of living parietal cells, although frequently discussed by those interested in gastric physiology, has not been available(1). This communication reports a technic that provides a suspension of living parietal cells. Although not pure, it is a good working model with which to pursue further purification of the preparation as well as to study the physical and chemical activities of this unique cell.

Material and method. Gastric mucosa from young rabbits is used because of the facility with which it lends itself to this technic, although the procedure yields almost equally good results with dog and human gastric mucosa. The rabbit is sacrificed with sodium pentobarbital, the gastric fundus immedately removed, rinsed in a salt solution (GKN) and the mucosa manually stripped from submucosa and muscularis. The tissue is then minced with scissors, rinsed again with the salt solution and placed in an extraction flask for digestion in collagenase for 2 hours at 37°C. After rinsing, the suspension is strained through an ordinary blood-plasma administration set filter, rinsed several times and layered on gradient density media for differential centrifugation. The gradient is a long chain sucrose polymer (*FICOLL, Pharmacia Laboratories) prepared in advance by layering upon one another solutions of FICOLL of specific gravity 1.07, 1.05 and 1.02 respectively. The preparation is centrifuged for 12 minutes at 1000 rpm, the cells of each layer removed and rinsed. Smears of the various fractions are made by transferring material to a slide with a pipette, air drying and fixing in appropriate solutions for staining.

Cell survival is determined by trypan blue staining. Tetrazolium salts are used to stain for succinic dehydrogenase using the method

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TABLE I. Pepsin Concentration Expressed as Units of Proteolytic Activity.

Whole cell	Fraction B	Fraction A		
		Immediate	1 hr after extraction	3 hr after extraction
3.14	.325		3.16	
	1.848		11.73	4.42
8.18		2.882	6.25	2.616
4.15	1.01	2.19	4.23	2.209
	2.337	2.895	5.98	3.0
5.81	1.119	2.79	2.665	2.65
4.89	.238	1.575	5.01	1.594
12.35	1.852	2.717	4.22	1.967
2.85	2.32	2.059	4.55	2.523
6.25	.29	2.069	2.42	2.65

of Nachlas *et al.*(2), and hematoxylin and eosin and the Zimmerman trichrome stain employed for cell-type identification. Pepsin is determined by a modification of the Anson-Mirsky technic and reported in units of proteolytic activity.

Results. Suspensions of material from the lower fraction (termed fraction A) contain variable amounts of debris and a large number of distinct, intact, single cells (Fig. 1). The vast majority of the cells appear to be parietal cells. They are round or cuboidal, have a central nucleus, contain cytoplasmic granules and stain characteristically with hematoxylin and eosin. There are no zymogen (chief) cells identified in the preparations. The upper fraction (termed fraction B) contains cellular debris and few formed cells. Six grams (wet weight) of tissue yield between 10^6 to 3×10^7 cells per ml of suspension. There is an average of 60% survival after differential centrifugation as determined by trypan blue viability studies and 30 to 50% of all cellular elements appear to be parietal cells. The remainder are mostly leukocytes and connective tissue cells.

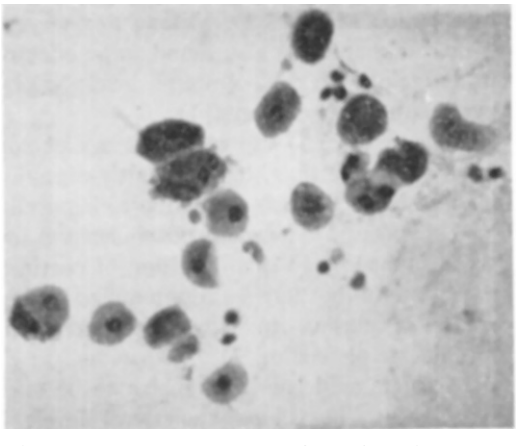

FIG. 1. An average suspension of parietal cells stained with nitro-BT (tetrazolium salt) for succinic dehydrogenase. The large, granular cells are parietal cells. The small cells and clump of cells and debris (left of center) take the counter stain and are probably leukocytes.

Of the cells found in the gastric mucosa, parietal cells have the capacity to give a strongly positive reaction for succinic dehydrogenase(3). The tetrazolium dye is taken up by the cytoplasm and stains a characteristically blue-black color. Suspensions so stained immediately after extraction show approximately 30-50% of the "cells" to be parietal cells. Within 4 hours after extraction the number of cells showing this positive reaction is markedly reduced and the colors seen are pale. Pepsin concentration in the lower fraction is equal or greater than in fraction B, but usually less than peptic activity determined in the whole cell preparation prior to centrifugation. After one hour of incubation at 37°C proteolytic activity rises sharply and at 3 hours falls to, or below, the level of the whole cell preparation.

Discussion. Failure to purify the suspension until there are only parietal cells present may be attributed to several factors. The most pressing are the problems of contamination with mucus, cellular debris accumulated during manual destruction of the tissue (mincing) and the tendency for cells to clump. The latter was not alleviated by EDTA, trypsin, chymotrypsin or dextran of low, intermediate or higher molecular weights, nor by ribonuclease. Overnight trypsinization in the cold (4°C) produces a somewhat

higher yield of single cells but also reduced the survival. Passing the suspension through an ordinary blood administration filter reduces the mucous contamination and does not substantially reduce the eventual yield.

Proteolytic activity persists in the preparation after differential centrifugation in the absence of stained zymogen (chief) cells probably because the pepsin molecule comes down almost equally well through all layers of the media although the chief cells do not. It is possible that the cytolysis liberates salts which significantly increase the solubility of pepsinogen. Another consideration is that the parietal cells might be coated with pepsinogen which is lost to the suspension solution during incubation and autolysis.

Gastric mucosal cells have been grown in our laboratory in continuous cell culture on well defined media through 14 passages whereupon contamination of the nutrient media forced us to discard the entire preparation and begin anew.

Summary. A technic that provides a suspension of living parietal cells is described. Gastric mucosa is minced, extracted with collagenase and the cells separated by differential centrifugation. Identification of cell types is achieved by histological and histochemical technics. Difficulties encountered in purification are discussed. The suspension is suitable for metabolic studies of the parietal cell.

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Effect of Hypocholesterolemic Agents on Intestinal Cholesterol Absorption.* (28087)

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Recently, several compounds, administered by the oral route, have been reported to be effective in lowering blood cholesterol levels in man and experimental animals. Stern and Treadwell† observed that cholesterol trimethylacetate, a branched-chain fatty acid ester of cholesterol, inhibited hydrolysis of cholesterol butyrate by pancreatic cholesterol esterase *in vitro*. It was also found that this branched-chain ester reduced blood cholesterol levels in rats when added to a diet containing cholesterol butyrate. According to Vahouny and Treadwell(1), cholesterol trimethylacetate itself is not absorbed in lymph-fistula rats and is not hydrolyzed by intraluminar cholesterol esterase.

Tennant *et al.*(2,3) have reported that MK-135 (cholestyramine), a bile acid-binding polymer, causes a marked reduction of blood cholesterol levels and a lower incidence of atheromatosis in cockerels. No toxic effects, either local or systemic, were detected in dogs fed MK-135 for as long as one year. This compound was also found to be effective in reducing blood cholesterol levels in hypercholesteremic patients(4).

Keys *et al.*(5,6) reported that a diet containing undigestible, or difficultly-digestible polysaccharides, such as cellulose, hemicellulose, or pectin, reduced blood cholesterol levels in humans. In rats, addition of pectin to a cholesterol diet largely counteracted any increase in plasma and liver cholesterol due to cholesterol feeding(7).

Nicotinic acid has been shown to be effective in lowering blood cholesterol levels in rabbits and humans; however, nicotinamide did not have a comparable effect(8,9). A study of the effect of nicotinic acid on hepatic synthesis of cholesterol showed a reduc-

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