Paper Electrophoretic Study of Protein-Losing Secretions from Explanted Gastric Mucosa in Rats.* (26688)

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Application of paper electrophoresis to analysis of the normal human gastric juice has resulted in identification of 8 to 11 large molecular components(1,2).

The most anodic of the components, P, represents pepsin, while the central electrophoretic components are mucoproteins which contain proteins or proteoses, hexoses, hexosamine, fucose, uronic and sialic acids as well as sulfates, and stain with amido black 10B, SF light green and periodic acid Schiff stains (3,4). Of the latter group, the most anodic one (glandular mucoprotein(5)) is supposedly derived from the fundic glands, while the central electrophoretic components, M_2, M_3, M_4 and X_1, represent mucous materials and their degradation products, derived from surface epithelium mucus and also, possibly, from the pyloric glands(2,6,7). The materials on the extreme cathodic side of the electrophoretic partition are large molecular peptides, probably derived from enzymic degradation of serum proteins(8).

In the search for *direct* information on cellular origin of these large molecular components of gastric juice, we used the method described by Toovey, Heller and Webster(9) and Webster, Toovey and Skoryna(10) for exteriorization of a portion of the rat glandular stomach onto the anterior abdominal wall. Histological studies(9,10) had demonstrated that after a lapse of a few weeks, the surface epithelium of the exteriorized gastric flap completely covered the surface of the explant, effectively blocking the orifices of the gastric glands. This enabled these investigators to obtain secretions from the gastric mucosa free of gastric glandular components. A preliminary abstract of our investigations has been published(11).

Method. The studies were carried out on adult male rats of the Royal Victoria Hospital strain, a) with ligated cardia and pylorus, and b) with gastric explants.

Rats with cardia and pylorus ligation. The animals were starved for 12 hours, then anesthetized with nembutal. After irrigation of the stomach with normal saline to remove food remnants, the cardia was ligated at the junction of esophagus and forestomach. The pylorus was then ligated and the laparotomy wound closed by continuous suture. Care was taken that the vagus nerve and blood vessels were not included in the ligature. After 24 hours, the animals were again laparotomized. At this time, the gastric pouch enclosed between the cardiac and the pyloric ligatures usually bulged with secretions. The esophagus and duodenum were transected beyond the ligatures, the gastric pouch removed and, through a small incision in the forestomach, the secretion was emptied into a beaker. Eleven pools, each of 14 to 22 cc, were obtained, each representing the 24-hour secretion of several rats. Only pools of perfectly clear gastric juice, free of blood, were analyzed.

Rats with explanted gastric wall. The animals were starved for 12 hours, then anesthetized with nembutal. A portion of the anterior and posterior gastric walls adjoining the greater curvature was separated into a flap with maintained blood supply. The continuity of the stomach was then restored by closing the transection line with double continuous suture. The flap of the glandular stomach was then exteriorized and the abdominal wall closed around the mesentery, care being taken not to impair the blood supply of the flap. The serosa of the flap was then sutured to the skin of the anterior abdominal wall.

Following explantation, progressive inflammation and destruction of the explanted mucosa was observed. After the second week,

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a new, low cuboidal epithelium could be seen, extending from the remaining intact mucosa at the mucocutaneous junction of the infolded edges of the flap. This newly-formed surface epithelium was almost entirely covered by a heavy lining of mucus. The surface not covered by epithelium and mucus was fibrous, or exhibited chronic ulcerations due to repeated traumatization of the exposed gastric surface. As shown by microscopic study, the deep glandular elements were retained, and often proliferated into the lamina propria. The ducts of the glands were dilated, however, since their orifices were blocked by the newly-formed surface epithelium. This rapid epithelialization was probably due to the higher division rate of surface epithelial cells as compared with mucous neck cells(12). The glandular elements of the mucosa in the explants thus either underwent atrophy or their orifices were shut off by new surface epithelium. After 4 weeks, the explant usually measured 1 × 1 cm. The animals were placed in Bollman cages and the fluid secreted from the flaps was collected in a glass cup or funnel, beginning 8 weeks after explantation.

The rats were kept on a standard Purina meal diet, with water *ad libitum* except for the starvation period. Total number of rats with explanted gastric mucosa, the secretions of which could be used for the present experiment, was 12. From each rat 10 to 12 collections were obtained over a period of about a month, volume of each animal's secretion totalling from 10 to 13 cc. Each collection was immediately centrifuged and the supernatant stored in a refrigerator. After pooling, total collection from each rat was divided into 2 portions. One was frozen and the other was extensively dialyzed at 4°C for 24 hours against frequent distilled water changes, then lyophilized. The same procedures were applied to the processing of the secretions of the stomachs with ligated cardia and pylorus.

The frozen native gastric juices were used for determination of HCl, pepsin, mucoprotein and mucoproteose, while the dialyzed and lyophilized juices were used for paper-electrophoresis. Pepsin activity was determined at pH 1.5 by modification of the hemoglobin method(13)·; cathepsin, by the same method, at pH 3.5; mucoprotein and mucoproteose, by the method of Glass and Boyd(14), and paper-electrophoresis of the gastric juice by the method of Glass, Stephanson and Rich(2), using Spinco cell, borate buffer of pH 9.0 and ionic strength 0.24, Whatman No. 1 paper, 0.4 mA/cm at 120 volts, for 5½ hours. The strips were stained with amido black 10B(2), SF light green (15) and PAS(16), then traced and integrated in Analytrol, using 575 mμ filter for amido black, 620 mμ for SF light green, and 550 mμ for the PAS stain.†

Results. Chemical assays of gastric secretion. The gastric juices from the ligated rat stomachs contained acid and pepsin, as shown in Table I. Gastric juices from the explants, however, contained no free HCl, pepsin or 'cathepsin'. 'Glandular mucoprotein' was either absent, or present in traces (0 to 9.3 mg/100 ml), in agreement with Webster *et al.*(10). On the other hand, gastric 'mucoproteose', which is known to be derived from the surface epithelium(17,18) was present in all these collections, its concentration ranging from 15.9 to 62.1 mg (mean: 31.1 mg/100 ml). These values were slightly below those obtained by Webster *et al.*(10) on the secretions of rat gastric mucosa explanted by the same method (mean: 49.2 mg/100 ml).

Our results have confirmed the fact that the explant technic in rats(9,10) achieves a situation comparable to that encountered in advanced gastric atrophy in humans(5,7,17, 18).‡ None of the glandular products (*i.e.*, HCl, pepsin, 'cathepsin' or 'glandular mucoprotein') are present here in appreciable amounts. Only gastric 'mucoproteose' was detectable, again confirming the derivation of this material from the surface epithelium (14,17,18), a view corroborated by other investigators(10,19).

† The technical assistance of Sallie E. Mitchell and Marilyn Rich in these chemical and electrophoretic studies is here gratefully acknowledged.

‡ A similar dedifferentiation and atrophy of the gastric mucosa has recently been reported in dogs, with use of the exteriorization technic(25).

TABLE I. Composition of Rat Gastric Juices and Secretions from Explanted Rat Gastric Mucosa.

	No.	No. of rats	Total vol, ml	Free acid	Pepsin, $PU_{Hb} \times 10^{-4}$/ml	Cathepsin, $CU_{Hb} \times 10^{-4}$/ml	Glandular muco-protein, mg/100 ml	Muco-proteose, mg/100 ml
Pooled gastric juices*	1	7	20	Present	19.9			
	2	8	22	"	12.5			
	3	4	14	"	9.4			
	4	6	16	"	7.5			
	5	5	15	"	6.7			
Secretions of gastric mucosal explants†	A-1	1	11.4	Absent	0	0	1.3	22.9
	2	1	12.0	"	0	0	9.8	63.7
	3	1	10.0	"	0	0	4.0	28.0
	4	1	13.0	"	0	0	4.4	21.2
	5	1	12.2	"	0	0	5.5	72.1
	6	1	10.0	"	0	0	2.6	25.4
	B-1	1	2.5	"	0	0	0	15.9
	3	1	2.0	"	0	0	0	19.9
	4	1	2.8	"	0	0	0	19.1
	5	1	4.2	"	0	0	trace	25.5
	6	1	3.5	"	0	0	0	28.5

* Each pool was obtained by pooling the 24-hr secretions of stomachs, ligated at cardia and pylorus, of from 4 to 8 rats.

† Secretion from each rat, A-1 to A-6, represents total of 10-12 continuous collections from the explant over a period of one month. Secretions from rats B-1 to B-6 represent materials collected over 5-7 days.

Paper-electrophoretic analysis of explant secretions. Electrophoretic patterns of normal rat gastric juice (Fig. 1) are similar to those of humans (Fig. 2) except for components Y and Z, which, in rat juice, are either absent or present in very low concen-

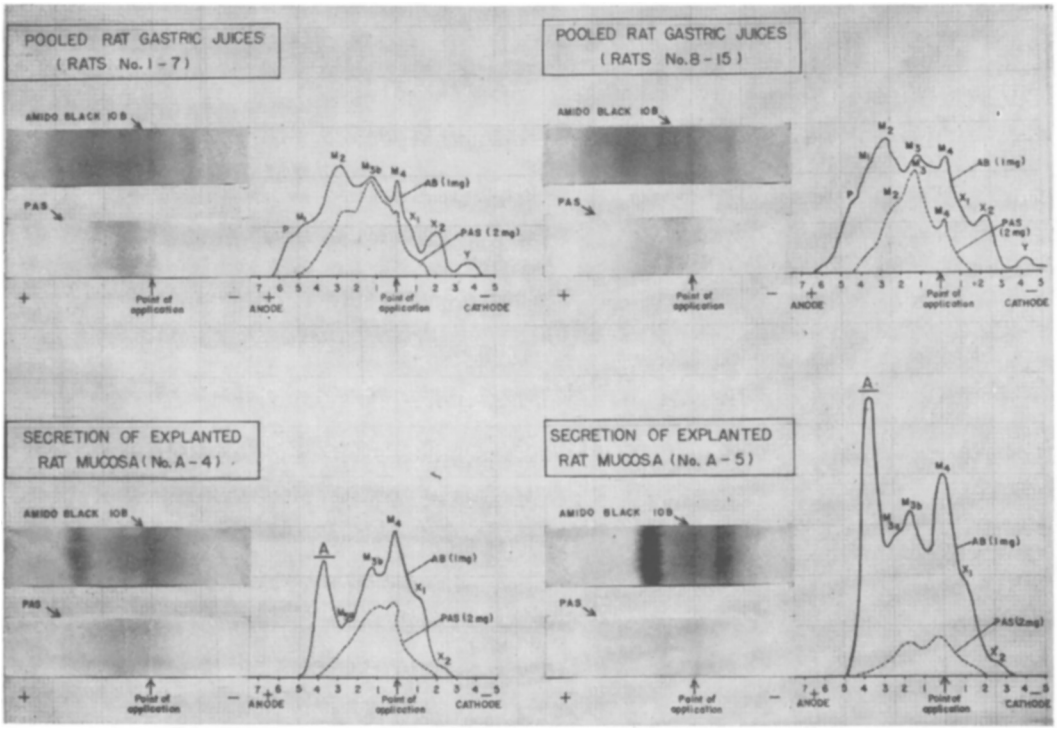

FIG. 1. Paper electrophoretic patterns of pooled normal gastric juices and secretions from explanted gastric mucosa of rats. (Amido black 10B and PAS stains.)

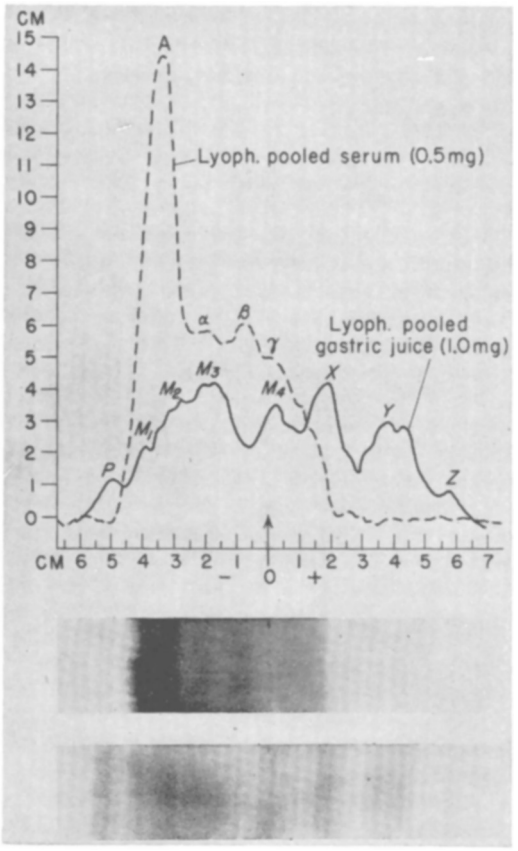

FIG. 2. Paper electrophoretic patterns of normal gastric juice and serum in man. (Amido black 10B stain.)

tration. Electrophoregrams of the secretion from the explanted mucosa differ markedly, however, from those of the normal rat gastric juice by absence of pepsin (P), glandular mucoprotein (M_1), the cathodic component X_2, and by high concentration of components M_3 and M_4 (Fig. 1). This agrees with the findings of Webster *et al.*(10) and others, and with the data given above. Further, these electrophoretic studies point again to the surface epithelial origin of gastric mucoproteose (14,17-19) and its electrophoretic correlates, components M_3 and M_4 (2,3,6).

The most marked abnormality of the secretion from rat explanted mucosa is the presence of a large component, labelled "A", which has a mobility similar to that of mucous substance M_2, but differs from it by staining heavily with amido black 10B and only slightly with carbohydrate-staining PAS (Fig. 1). Identical patterns were obtained for other explants (Fig. 3). This component has the electrophoretic mobility and staining properties of serum albumin as shown by Fig. 2 which includes paper-electrophoretic tracings of normal human gastric juice and serum.

This albumin-like material shows other features also, reported elsewhere(20,21) which speak for its identity with serum albu-

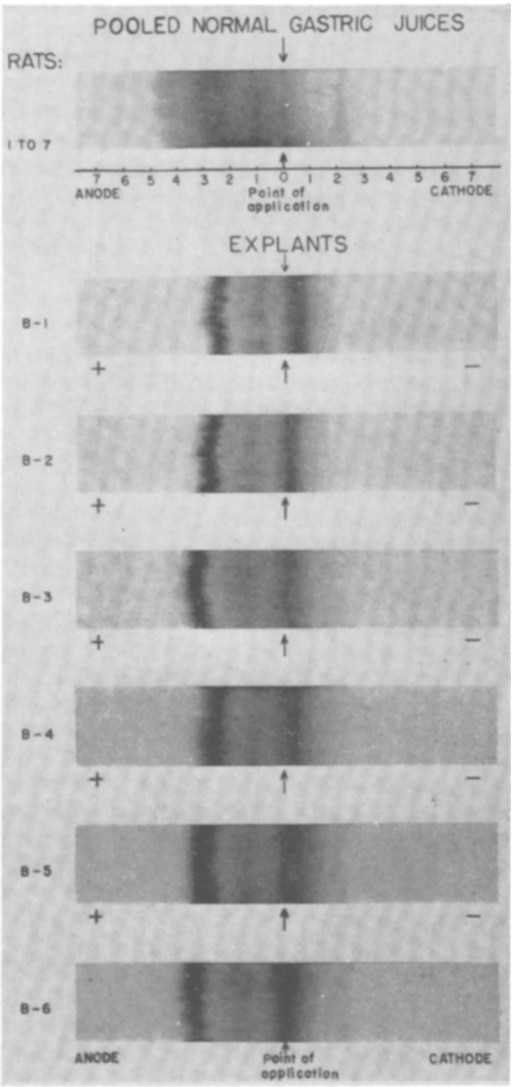

FIG. 3. Paper electrophoretic patterns of pooled normal gastric juices of 7 rats and of 6 explants of rat gastric mucosa. (Amido black 10B stain; 0.4 mg of dialyzed and lyophilized materials applied to the center of the strips.)

min. These are: 1) Precipitability with trichloracetic acid. 2) An electrophoretic mobility of the trichloracetic acid precipitate identical to that of serum albumin at pH 7 to 9. 3) Solubility of this trichloracetic acid precipitate in 80% acetone and 95% alcohol, a unique feature of serum albumin precipitate.

In many electrophoregrams of explant secretions an additional component may be detected with amido black 10B (Fig. 1) and SF light green (not shown in Figure), but which stains very little with PAS and is located slightly to the cathodic side of the application point in the area of the M_4 band. This component probably represents gamma globulin which has passed (from the blood serum) into the secretion of the mucosal explant.

Discussion. Secretions from the explanted rat gastric mucosa are electrophoretically similar to those of the gastric juices of patients with protein-losing gastropathies. We have previously reported similar electrophoretic tracings in the anacid gastric juices of patients with gastric cancer, atrophic gastritis, pernicious anemia and Menetrier's disease(6, 8,20). Secretions from all the rat explants show a similar albumin-like band. Most of them also contain a large component in the area of the M_4 band which probably represents serum gamma globulin. The presence of these serum protein-like materials in secretions of the explanted mucosa is not due to an admixture of blood, as proven by its absence from collections and no hemoglobin band in the electrophoregram of the gastric juice, the usual location of which is, with our technic, slightly to the anode from the application point.

The explanted rat gastric mucosa probably leaks serum proteins in a way similar to that of the gastric mucosa of patients with protein-losing gastropathies(22,23). Leakage of serum proteins through the explanted gastric mucosa of rats may be due both to mucosal atrophy(6) and ulcerations which exude serum proteins. The absence of acid and pepsin in these secretions prevents degradation of albumin, thus making its detection possible in the electrophoretic tracing(8,21,24).

Summary and conclusions. Gastric secretions were collected from the stomachs of rats with ligated cardia and pylorus and from the explanted gastric flaps of male rats. Explantation of gastric flaps in male rats results in atrophy of the superficial elements of the gastric glands, obliteration of glandular ducts by overgrowth of cuboidal surface epithelium, and fibrosis and superficial ulceration of the explanted mucosa.

The absence of all glandular components of gastric juice from the secretion of the explants, as previously reported by other workers, was confirmed. This was true of HCl, pepsin, 'cathepsin' and 'glandular mucoprotein' and their electrophoretic correlates, components P and M_1, as well as of the cathodic component X_2. Conversely, the presence of gastric mucoproteose and, on paper electrophoresis, the corresponding electrophoretic band M_3, confirms the surface epithelial origin of these materials. The electrophoretic patterns of secretions from explanted gastric mucosa are similar to those observed in gastric juices of patients with protein-losing gastropathies in that they contain a serum albumin-like component which stains heavily with amido black 10B and SF light green stains, but not with PAS. The presence of serum protein-like materials in explant secretions is not due to an admixture of blood, but is probably due to transudation of serum proteins through the atrophic mucosa, or exudation of serum from superficial ulcerations which have developed in the explanted mucosa, or to both of these factors.

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A Method for Obtaining Monolayer Cultures of Human Fetal Cells from Term Placentas.* (26689)

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The human placenta at term is considered by most authorities to be a senescent organ and histological preparations show extensive fibrinoid degeneration, calcification and infarction(1,2,3,4). In previous years investigators have reported failures in their attempts to grow cells from cultures of the villi of mature placentas(5,6). More recently Stewart, Sano and Montgomery(7), using explant cultures, obtained an outgrowth of fibroblasts from the villous portion of the mature placenta, but they were unable to grow the trophoblast or to detect in the growth medium any of the gonadotrophic hormone that is produced by the trophoblast *in vivo*. However, we found, while studying cultures of cells from placentas procured at time of therapeutic abortion, that it is also possible to obtain vigorous healthy cultures of a mixed cell type from the villi of mature placentas. In some cases chorionic gonadotrophin was demonstrated. Because of the shortage of human embryonic tissue for investigative work, the possibility that the villous portion of the mature placenta might provide an additional source of fetal cells was further explored. This paper describes a uniformly reliable technic for obtaining monolayer cultures of human fetal cells from the chorionic villi of mature placentas and describes the methods used to identify these cells as being of fetal origin.

Methods and materials. (A) *Tissue culture.* Mature placentas from 14 male and female pregnancies were collected aseptically at birth. Most of these were delivered vaginally. Individual cotyledons were identified on the maternal surface, excised and placed on a sterile towel with the fetal surface up. The membranes were removed exposing fetal tissue, and a layer containing chorionic villi

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