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THE ORIGIN OF IRON CHELATING FACTORS IN GASTRIC JUICE

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Introduction

THE concept that gastric juice contains a component called "Gastroferrin" which is involved in iron absorption was put forward by Davis *et al.* (1966); this was subsequently criticised by Wynter and Williams (1968 a, b). It is clear, however, that gastric juice does bind iron in vitro and that the unusual characteristics described by Wynter and Williams (1968 a) are still compatible with true binding (Morgan *et al.*, 1969). The problem remains as to the physiological significance of the 'iron binding capacity' (I.B.C.) and the source of its secretion from the gastric mucosa.

We have previously observed that individual subjects have shown a marked degree of fluctuation of the iron binding capacity of gastric secretion obtained in response to continuous histamine infusion, under conditions where both the volume and acid content of the gastric secretion are constant (Lawrie *et al.*, 1964). This suggested that the secretion of the iron binding capacity (I.B.C.) was irregular and probably not coming from the gastric mucosal cells directly under the influence of vagal stimulation. This communication investigates the possibility that the I.B.C. of gastric juice may arise from the mucous cells of the gastric mucosa.

Patients and Methods

Gastric secretion was obtained from subjects with duodenal peptic ulcers. The method of gastric stimulation with a continuous histamine infusion (40 μg/kg body wt./hour), of collection of gastric secretion and estimation of the acid content was that of Lawrie *et al.* (1964) with the following modifications. Gastric secretion was first collected over a basal hour, the basal secretion was then allowed to collect in the stomach for a further ten minutes, this fluid was flushed in and out of the stomach for 3-4 minutes, using a 20 ml. syringe and labelled "flushed gastric secretion". Following this histamine was infused for two hours and gastric secretion collected in the usual way. The iron chelating ability of the gastric juice was determined as previously described (Morgan *et al.*, 1969) at various concentrations of iron and the maximum iron binding point was used in the tabulation of the results. Where the pH of the gastric juice was not acid to begin with, it was adjusted to pH 2.0 with 1.0 N. Hcl.

TABLE 1

PATIENT		Basal Hour	First Post Histamine Hour	Second Post Histamine Hour	Flushed Gastric Secretion
C. O'H	1	30	80	60	90
	2	115	96	96	21
	3	5.65	99.5	127.3	6'5
P. C	1	90	70	60	110
	2	40	240	190	45
	3	33.25	90	116	46.6
J. K	1	120	100	120	180
	2	24	51	24	12
	3	1.1	6.6	16.6	3.4
P. F	1	50	50	40	120
	2	120	125	250	17
	3	28.3	40.3	63.2	31.7
T. W	1	60	40	140	160
	2	93	350	520	35
	3	43	73.5	76.6	51.4
A. B	1	100	90	70	140
	2	42	38	85	18
	3	0.7	1.3	44.7	0.4
D. O'B	1	100	60	90	130
	2	24	51	24	12
	3	6.2	110	118	5.8
E. C	1	60	90	80	140
	2	105	245	130	36
	3	50	72	66.6	46.6

Iron binding, volume and acid concentration under various methods of collection of gastric juice. Maximum iron binding capacity mμ moles of iron bound per ml of gastric juice (1); total volume of secretion in mls (2); hydrogen iron concentration in mille equivalents per litre of gastric juice (3).

Results

The results of the maximum iron binding capacity of gastric juice collected under various conditions are shown in Table 1 and Fig. 1. The ratios of the iron binding capacity of the 1st and 2nd post histamine hours and the "flushed gastric secretion" to the basal value obtained in each patient were calculated. The mean ratios were 1.14 (±0.26 S.E.), 1.14 (±0.16 S.E.) and 1.93 (±0.25 S.E.) respectively. There was no significant difference of the I.B.C. in the 1st and 2nd post histamine hours from unity. However, the corresponding ratio of the binding capacity obtained from the "flushed gastric secretion" was highly significant ($P<0.01$).

To ensure that the flushing procedure used in the above experiment did not affect the histamine stimulated results, histamine stimulation was also carried out immediately after the collection of the basal hour (Table 2). Again no significant difference was found in the I.B.C. between the basal and histamine stimulated secretion.

Discussion

Following continuous histamine stimulation over a two hour period the mean concentration of the iron binding capacity (I.B.C.) in gastric secretion was on average of the same order as that obtained in the basal hour secretion (Table 1). This suggests that either the I.B.C. was stimulated by the histamine infusion to the same extent as the volume of gastric secretion, or that the I.B.C. was washed out with the increased fluid secretion. The constituent of gastric secretion which is most likely to fit these criteria is mucus. Webster (1967) showed that mucus secretion in dogs following a subcutaneous injection of histamine increased approximately in proportion to the volume increase, and suggested that this was a form of "wash out" effect.

Mucus secretion from the stomach is known to increase following mechanical stimulation by gentle rubbing of the gastric mucosa (Hollander, 1962); in this instance mucus secretion was stimulated by "flushing" the basal gastric secretion against the gastric mucosa. This form of stimulation leads to a marked increase in the I.B.C. (Fig. 1), suggesting that its origin is the mucus secreting cells of the stomach. It is impossible at this stage to state whether one or all of the mucus secreting cells are responsible for the secretion of the I.B.C., although this form of stimulation is more likely to stimulate the surface mucous cells than the mucous neck cells. It is also unlikely that the I.B.C. results from cellular debris as this would have been removed by the centrifugation of the gastric secretion at 18,000 x g. The I.B.C. has been shown to be basically a carbohydrate (Morgan *et al.*, 1969; Rudzki and Deller, 1969) which is compatible with the I.B.C. being of mucus origin.

In this regard it is interesting to note that mucopolysaccharides are known to have a marked affinity for iron (Hale, 1946) particularly at low pH (Pearse, 1960), and this is made use of in Hale's method of staining for acid mucopolysaccharides. The fact that mucin binds iron at low pH especially may explain why the I.B.C. of gastric secretion will only bind iron when both are mixed at low pH before being neutralised (Jacobs & Miles, 1969).

McCarthy *et al.* (1964) has demonstrated that a variety of mucins injected into the lung, and taken up by macrophages, have a particular affinity for iron which is probably derived by sequestration of iron circulating in the blood. This finding may be relevant to the aetiology of pulmonary haemosiderosis.

The existing evidence would thus seem to indicate that iron binding is due to mucus type secretion but there is nothing to suggest that gastric mucus has a greater affinity for iron than any other mucus. Furthermore, while it is possible that a single mucoid substance is responsible for binding iron it would seem more probable that most if not all gastric mucus secretion is involved. It therefore seems inappropriate to use the name 'gastroferrin' in this context as it implies a single component which has as its function, the binding of iron. We suggest that it would be more appropriate to refer instead to the iron binding capacity (I.B.C.) of gastric juice.

Summary

Comparison of the iron binding capacity of gastric secretion under basal

TABLE 2

PATIENT		Basal Hour	First Post Histamine Hour	Second Post Histamine Hour
J. H	1	70	20	20
	2	115	193	245
	3	5.65	99.5	127.3
G. D	1	60	50	40
	2	102	170	215
	3	40	83.8	130.4
F. T	1	100	50	100
	2	96	460	560
	3	100	121.7	127
E. W.	1	50	50	40
	2	135	235	250
	3	76.6	136	129.6
M. M	1	60	60	70
	2	27	240	190
	3	36.6	90	102

Iron binding, volume and acid concentration under various methods of collection of gastric juice. Maximum iron binding capacity mμ moles of iron bound per ml of gastric juice (1); total volume of secretion in mls (2); hydrogen ion concentration in mille equivalents per litre of gastric juice (3).

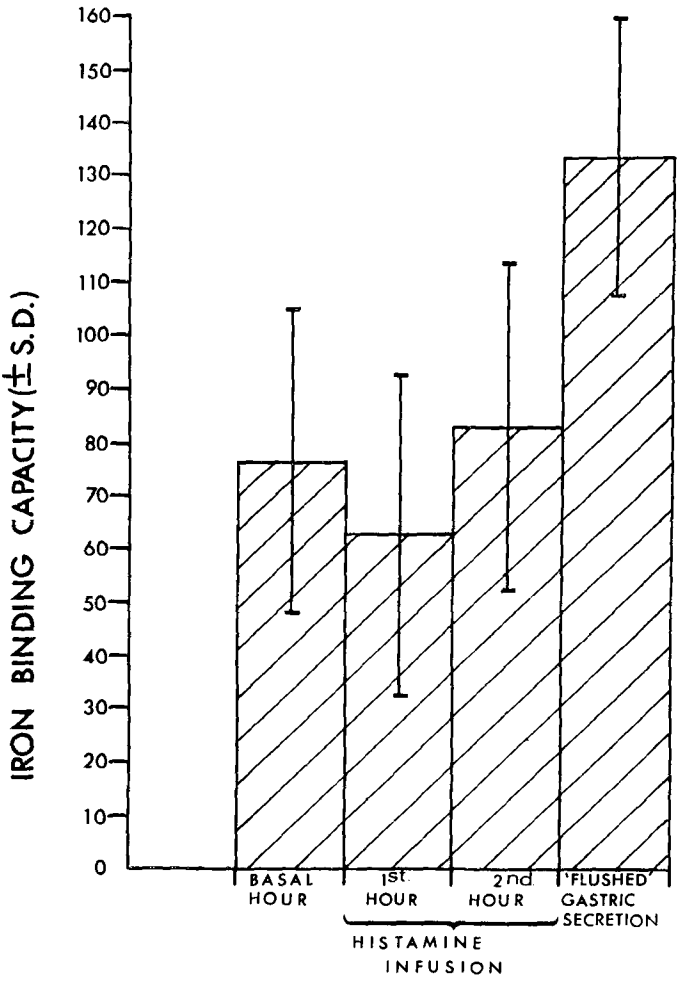

Fig. 1—Mean iron binding capacity of the gastric secretion under varying conditions in eight subjects.

and histamine stimulated conditions showed no significant variation. However, following 'flushing' of the gastric mucosa the capacity to bind iron was approximately doubled. Since this procedure is known to cause increased mucus secretion it seems likely that the iron binding capacity in gastric juice is either of mucoid origin or associated with mucus secretion. From what is known of the properties of gastric iron binding the former seems to be the more likely. Furthermore since there is no evidence that iron binding in gastric juice is due to a single component it seems more appropriate to refer to 'the iron binding capacity' of gastric juice than to imply by the use of the name 'Gastroferrin' that a single entity is involved.

References

Davis, P. S., Luke, C. G. and Deller, D. J. (1966). Reduction of gastric iron-binding protein in haemochromatosis. Lancet, 2, 1431-1433.

Hale, C. W. (1946). Histochemical demonstration of acid polysaccharides in animal tissues. Nature, 157, 802-803.

Hollander, F. (1962). The physiology and chemistry of the secretion of gastric mucus. Gastroenterology, 43, 304-309.

Jacobs, A., Miles, P. M. (1969). Role of gastric secretion in iron absorption. Gut, 10, 226-229.

Lawrie, J. H., Smith, G. M. R. and Forrest, A. P. M. (1964). The histamine infusion test. Lancet, 2, 270-273.

McCarthy, C., Reid, L. and Gibbons, R. A. (1964). Intra-alveolar mucus-removal by macrophages : with iron accumulation. J. Path. Bact., 8, 39-47.

Morgan, O. S., Weir, D. G., Gatenby, P. B. B. and Scott, J. M. (1969). Studies on an iron-binding component in human gastric juice. Lancet, 1, 861-863.

Pearse, A. G. E. (1960) in Histochemistry, theoretical and applied 2nd ed., London, p. 259.

Rudzki, Z. and Deller, D. J. (1969). Iron binding component in human gastric juice. Lancet, 1, 1096.

Webster, D. R. (1967) in Gastric Secretion edited by Shnitka, J. K, Gilbert, J. A. L. and Harrison, R. C. Pergamon Press, London, 1st ed., p. 214-224.

Wynter, C. V. A. and Williams, R. (1968a). Iron binding properties of gastric juice in idiopathic haemochromatosis. Lancet, 2, 534-537.

Wynter, C. V. A. and Williams, R. (1968b). Gastric iron binding in haemochromatosis. Lancet, 2, 1243.