

24-5

MOTILIN MEASUREMENT AND ASPECTS OF ITS NATURE,
PHYSIOLOGY AND PATHOPHYSIOLOGY

NICOS DEMETRIOU CHRISTOFIDES

Thesis submitted for the degree of Doctor of Philosophy

ROYAL POSTGRADUATE MEDICAL SCHOOL, UNIVERSITY OF LONDON

DEDICATION

I dedicate this thesis to my wife and son and my parents.

ABSTRACT

Motilin is a straight chained pharmacologically active peptide, with an amino-acid sequence different from other known hormones. It has a broad spectrum of motor actions in the gastrointestinal tract.

A precise and specific radioimmunoassay has been developed, which is able to detect changes in plasma motilin of 3pmol/l. Regionally specific antibodies were used to characterise motilin, immunologically, in several species, and to determine its release, physiology and pathophysiology in man.

Motilin was found to be localised exclusively to the jejunum, duodenum and antrum, though only in small amounts in the latter. Chromatographic analysis of human intestinal extracts and plasmas revealed the presence of two forms of motilin immunoreactivity. The earlier eluting form, of apparently higher molecular weight, had a similar gross distribution along the intestine as the smaller form. The use of C and N terminally reacting antibodies suggested that this 'big motilin' may contain the sequence of small motilin with an elongation at the C-terminal end.

Examination of plasmas following various stimuli indicated a parallel release of both forms of motilin. Infusion of porcine motilin resulted in a rise in plasma motilin concentrations of the smaller form (the exogenous motilin), whereas the larger form was decreased suggesting the presence of a negative inhibitory control mechanism.

Motilin was released following ingestion of food, the rise being small and transient. Different food components elicited different responses, with fat stimulating, and glucose inhibiting the release. Gastric distension, intravenous nutriments, and other hormones were shown to affect the release of motilin, exemplifying the complexity of the mechanisms involved.

Infusion of motilin in man, at a dose to reproduce 'physiological' concentrations demonstrated an acceleration of the gastric emptying of a mixed meal, liquid glucose and the stimulation of small intestinal activity.

It was of interest that patients with several different types of diarrhoea had elevated plasma motilin concentrations, above those found active in the infusion studies.

PUBLICATIONS

1. Mitznegg, P., Bloom, S.R., Christofides, N.D., Besterman, H.S., Domschke, W., Wunsch, E., and Demling, L., : Release of motilin in man. Scand J. Gastroent., (Suppl 39) 11:53, 1976
2. Christofides, N.D., Bloom, S.R., and Besterman, H.S., : Physiology of motilin. In : Bloom S.R. (ed). Gut Hormones, first edition, Churchill Livingstone, Edinburg, P343, 1978
3. Christofides, N.D., and Bloom, S.R., : Plasma motilin levels in duodenal ulcer and effect of truncal vagotomy and hypoglycaemia. Experientia (Basel), 34:809, 1978
4. Bloom, S.R., Mitchell, S.J., Greenberg, G. R., Christofides, N.D., Domschke, W., Domschke, S., Mitznegg, P., and Demling, L., : Release of VIP, secretin and motilin after duodenal acidification in man. Acta Hepato-Gastroent, 25:365, 1978
5. Christofides, N.D., Modlin, I.M., Fitzpatrick M.L., and Bloom, S.R., : Effect of motilin on the rate of gastric emptying Gastroenterology, 76:903, 1979
6. Christofides, N.D., Bloom, S.R., Besterman, H.S., Adrian, T.E., and Ghatei, M.A. : Release of motilin by oral and intravenous nutrients in man. Gut, 20:102, 1979
7. Vantrappen, G., Janssens, J., Hellemans, J., Christofides, N.D., and Bloom, S.R. : Studies on the interdigestive (migrating) motor complex in man. In : Duthie, H. (ed). Proc. VIth. Inter. Symp. Gastrointestinal Motility, MTP Press, Edinburgh, P3, 1978
8. Christofides, N.D., Sarson, D.L., Albuquerque, R.H., Adrian, T.E., Ghatei, M.A., Modlin, I.M., and Bloom, S.R. : Release of gastrointestinal hormones following an oral water load. Experientia (Basel), 35:1521, 1979

9. Vantrappen, G., Janssens, J., Peeters, T.L., Bloom, S.R., Christofides, N.D., and Hellemans, J., : Motilin and the interdigestive migrating motor complex in man. Dig. dis. Sci., 24:497, 1979
10. Besterman, H.S., Cook, G.C., Sarson, D.L., Christofides N.D., Bryant, M.G., Gregor, M., and Bloom, S.R., : Gut hormones in tropical malabsorption. Brit. Med. J., 1:1252, 1979
11. Adrian, T.E., Greenberg, G.R., Barnes, A.J., Christofides, N.D., Alberti, K.G.M.M., and Bloom, S.R., : Effects of pancreatic polypeptide on motilin and circulating metabolites in man. Europ. J. Clin. Invest., 10:235, 1980
12. Reverdin, N., Hutton, M.R., Ling, A., Thompson, H.H., Wingate, D.L., Christofides, N.D., Adrian, T.E., and Bloom, S.R. : Vagotomy and the motor response to feeding. In : Christensen, J., (ed). Gastrointestinal motility, Raven Press, New York, P359, 1980
13. Long, R.G., Albuquerque, R.H., Prata, A., Barnes, A.J., Adrian, T.E., Christofides, N.D., and Bloom, S.R., : Response of plasma pancreatic and gastrointestinal hormones and growth hormone to oral and intravenous glucose and insulin hypoglycaemia in Chagas's disease. Gut, 21:772, 1980
14. Blackburn, A.M., Christofides, N.D., Ghatei, M.A., Sarson, D.L., Ebeid, F.H., Ralphs, D.N.L., and Bloom, S.R., : Elevation of plasma neurotensin in the dumping syndrome. Clin. Sci., 59:853, 1980
15. Lucas, A., Adrian, T.E., Christofides, N.D., Bloom, S.R., and Aynsley-Green, A., : Plasma motilin, gastrin, and enteroglucagon and feeding in the human newborn. Arch. Dis. Child., 55:673, 1980
16. Rennie, J.A., Christofides, N.D., Mitchenere, P., Fletcher, D., Leathard, H., Bloom, S.R., Johnson, A.G., and Harding-Rains, A.J. : Neural and humoral factors in post-operative ileus. Br. J. Surg., 67:694, 1980

17. Blackburn, A.M., Fletcher, D.R., Bloom, S.R., Christofides, N.D., Long, R.G., Fitzpatrick, M.L., and Baron, J.H., : Effect of neurotensin on gastric function in man. Lancet, 1:987, 1980
18. Long, R.G., Barnes, A.J., Adrian, T.E., Mallinson, C.N., Brown, M.R., Vale, W., Christofides, N.D., and Bloom, S.R. : Suppression of pancreatic tumour secretion by long-acting somatostatin analogues. Lancet, II:764, 1979
19. Polak, J.M., Pearse, A.G.E., Szelke, M., Bloom, S.R., Hudson, D., Facer, P., Buchan, A.M.J., Bryant, M.G., Christofides, N.D., and MacIntyre, I. : Specific immunostaining of CCK cells by use of synthetic fragment antisera. Experientia (Basel), 33:762, 1977
20. Christofides, N.D. : Importance of the jejunal hormone motilin. J. Clin. Path., (Suppl 8), 33,51, 1980
21. Christofides, N.D., Bloom, S.R., Vantrappen, G., Janssens J., Peeters, T.L., and Hellemans, J. : Postprandial release of motilin in relation to the interdigestive motor complex in man. Biomedical Research, 2:67,1981
22. Christofides, N.D., Bryant, M.G., Ghatei, M.A., Kishimoto, S., Buchan, A.M.J., Polak, J.M., and Bloom, S.R. : Molecular forms of motilin in the mammalian and human gut and human plasma. Gastroenterology, 80:292, 1981
23. Christofides, N.D., Long, R.G., Fitzpatrick, M.L., McGregor, G.P., and Bloom, S.R. : Effect of motilin on the gastric emptying of glucose and fat in man. Gastroenterology, 80:456,1981
24. Christofides, N.D., and Bloom, S.R. : Parallel release of two molecular forms of motilin in human plasma and suppression of big motilin by exogenous motilin infusion. Biomedical Research, 1981, In Press

25. Christofides, N.D., and Bloom, S.R. : Motilin.
In : Bloom, S.R., and Polak, J.M. (eds). Gut Hormones, 2nd edn.
Churchill Livingstone, Edinburgh, P273, 1981
26. Greenberg, G.R., Wolman, S.L., Christofides, N.D.,
Bloom, S.R., and Jeejeebhoy, K.N. : The effect of total
parenteral nutrition on gut hormone release in man.
Gastroenterology, 1981, In Press
27. Dawson, J., Bryant, M.G., Cox, A.G., Christofides, N.D.,
Bloom, S.R., and Peters, T.J. : Subcellular localisation of
serotonin and motilin in the human jejunum. Clin. Sci.,
59:505, 1980
28. Christofides, N.D., Pancreatic Glucagon.
In : Bloom, S.R., and Long, R.G. (eds). Radioimmunoassay of
Gut Hormones, 1981, In Press
29. Christofides, N.D., Motilin. In : Bloom S.R., and
Long, R.G. (eds). Radioimmunoassay of Gut Hormones, 1981,
In Press
30. Christofides, N.D. : Assay Trouble-Shooting. In :
Bloom, S.R., and Long, R.G. (eds). Radioimmunoassay of Gut
Hormones, 1981, In Press
31. Besterman, H.S., Adrian, T.E., Bloom, S.R.,
Christofides, N.D., Mallinson, C.N., Ponti, V., Lombardo, L.,
Modigliani, R., Guerin, S., and South, M. : Pancreatic and
gastrointestinal hormones in chronic pancreatitis. Submitted
to Digestion
32. Besterman, H.S., Adrian, T.E., Bloom, S.R.,
Christofides, N.D., Sarson, D.L., Pera, A., Lombardo, L.,
Mallinson, C.N., and Modigliani, R. : Gut hormone release
following intestinal resection. In Preparation
33. Ghatei, M.A., Bryant, M.G., Christofides, N.D.,
Moody, A.J., and Bloom, S.R. : Molecular forms of glucagon-
like immunoreactivity in the porcine gut and pancreas.
In Preparation

34. Ghatei, M.A., Bryant, M.G., Christofides, N.D., Gregor, M., Dawson, J., Peeters, T.J., and Bloom, S.R. : Radioimmunoassay of human enteroglucagon : Characterization of Molecular forms in tissue and plasma and their location in a single subcellular storage granule type. In Preparation
35. Ghatei, M.A., Jung, R.T., Bloom, S.R., Hillyard, C., Lee, Y.C., Stevenson, J.C., Mashiter, K., Adrian, T.E., Sarson, D.L., and Christofides, N.D. : Effects of bombesin in man. In Preparation
36. Adrian, T.E., Mitchener, P., Sagor G.R., Christofides N.D. and Bloom, S.R. : Motilin-induced gall bladder contraction - A New mechanism. In Preparation
37. Rennie, J.A., Christofides, N.D., Johnson, A.G., and Bloom, S.R. : Motilin and colonic motility in man. In Preparation
38. Besterman, H.S., Christofides, N.D., Welsby, P.D., Sarson, D.L., and Bloom, S.R. : Elevated plasma motilin and enteroglucagon in patients with acute diarrhoea. In Preparation
39. Christofides, N.D., Ghatei, M.A., Gillmer, M.D.G., and Bloom, S.R. : Low plasma motilin in human pregnancy. In Preparation
40. Long, R.G., Christofides, N.D., Fitzpatrick, M.L., O'Shaughnessy, D.J., and Bloom, S.R. : Effect of motilin and somatostatin on glucose tolerance. Submitted to Diabetes
41. Bloom, S.R., Mitznegg, P., Domschke, S., Christofides N.D., Wunsch, E., and Demling, L. : Release of motilin in man. Gut, 17:816, 1976 (Abst)
42. Christofides, N.D., and Bloom, S.R. : Plasma motilin levels in duodenal ulcer and the effect of truncal vagotomy and hypoglycaemia. Gut, 18:406, 1977 (Abst)
43. Modlin, I.M., Bloom, S.R. and Christofides N.D. : Plasma Motilin in carcinoid tumours. Lancet 11:979, 1977 (Letter)

44. Bloom, S.R., Mitchell, S.J., Greenberg, G.R., Christofides, N.D., Domschke, W., Domschke, S., Mitznegg, P., and Demling, L. : Release of VIP by duodenal acidification in man. Gut, 18:963, 1977 (Abst)
45. Bloom, S.R., Mitznegg, P., Domschke, W., Domschke, S., Christofides, N.D., Wunsch, E., and Demling, L. : Release of motilin in man. Gastroenterology, 72:812, 1977 (Abst)
46. Christofides, N.D., Modlin, I.M., Sarson, D.L., Albuquerque, R.H., Ghatei, M.A., Adrian, T.E., and Bloom S.R. : The release of gastrin, PP, VIP and motilin after an oral water load and atropine in man. Clin. Sci. Molec. Med, 54:12, 1978 (Abst)
47. Besterman, H.S., Adrian, T.E., Christofides, N.D., Sarson, D.L., Bloom, S.R., Mallinson, C.N., Pera, A., South, M., Modigliani, R., and Guerin, S. : Gut hormone profile in pancreatic disease. Gut, 19:444, 1978 (Abst)
48. Christofides, N.D., Modlin, I.M., Fitzpatrick, M.L., and Bloom, S.R. : Effect of motilin on gastric emptying of solid meals in man. Gut, 19:436, 1978 (Abst)
49. Sarson, D.L., Christofides, N.D., Albuquerque, R.H., Adrian, T.E., Ghatei, M.A., Modlin, I.M., and Bloom, S.R. : Release of gastrointestinal hormones after and oral water load and atropine in man. Gut, 19:436, 1978 (Abst)
50. Christofides, N.D., Bloom, S.R., Besterman, H.S., Adrian, T.E., and Ghatei, M.A. : Release of motilin in man, by oral and intravenous nutriments. Gut, 19:436, 1978 (Abst)
51. Bloom, S.R., Besterman, H.S., Cook, G.C., Sarson, D.L., and Christofides, N.D. : Gut hormone profile in post-infective tropical malabsorption (acute tropical sprue). Gastroenterology, 74:1011, 1978 (Abst)

52. Bloom, S.R., Besterman, H.S., Adrian, T.E., Christofides, N.D., Sarson, D.L., Mallinson, C.N., Pera, A., Modigliani, R., and Guerin, S. : Gut hormone profile in pancreatic disease. Gastroenterology, 74:1012, 1978 (Abst)
53. Christofides, N.D., Bloom, S.R., Besterman, H.S., Adrian, T.E., and Ghatei, M.A. : Release of motilin by oral and intravenous nutriments. J. Endocr., 77:42, 1978 (Abst)
54. Bloom, S.R., Christofides, N.D., Modlin, I.M., and Fitzpatrick, M.L. : Effect of motilin on gastric emptying of solid meals in man. Gastroenterology, 74:1010, 1978 (Abst)
55. Bloom, S.R., Sarson, D.L., Christofides, N.D., Alberquerque, R.H., Adrian, T.E., Ghatei, M.A., and Modlin, I.M.: The release of gastrointestinal hormones following an oral water load and atropine in man. Gastroenterology, 74:1010 1978 (Abst)
56. Bloom, S.R., Christofides, N.D., Besterman, H.S., Adrian, T.E., and Ghatei, M.A. : Release of motilin in man by oral and intravenous nutriments. Gastroenterology, 74:1010, 1978 (Abst)
57. Polak, J.M. Buchan, A.M.J., Dryburgh, J.R., Christofides, N.D., Bloom, S.R., and Yanaihara, N. : Lancet, I:1364, 1978 (Letter)
58. Adrian, T.E., Besterman, H.S., Christofides, N.D., and Bloom, S.R. : Interaction of gastrointestinal hormones and cholinergic innervation on the release of pancreatic polypeptide. Scand. J. Gastroent. (Suppl. 49) 13 :1,1978 (Abst)
59. Bloom, S.R., Mitchell, S.J., Greenberg, G.R., Christofides, N.D., Domschke, W., Domschke, S., Mitznegg, P., and Demling, L. : The response of VIP, Secretin and motilin to duodenal acidification in man. Scand. J. Gastroent. (Suppl 49), 13:27, 1978 (Abst)

60. Bloom, S.R., Christofides, N.D., Besterman, H.S., Adrian, T.E., and Ghatei, M.A. : Release of motilin in man. Scand. J. Gastroent. (Suppl 49), 13:26, 1978 (Abst)
61. Adrian, T.E., Greenberg, G.R., Besterman, H.S., Christofides N.D., and Bloom, S.R. : PP infusion in man : pharmacokinetics at three dose levels and effects on gastrointestinal and pancreatic hormones. Scand. J. Gastroent. (Suppl 49) 13:3, 1978 (Abst)
62. Bloom, S.R., Christofides, N.D., and Besterman, H.S. : Raised motilin in diarrhoea. Gut, 19:959, 1978 (Abst)
63. Vantrappen, G., Janssens, J., Peeters, T., Bloom, S.R., Christofides, N.D., and Helleman, J. : Intraduodenal pH, motilin and interdigestive migrating motor complex in man. Scand. J. Gastroent. (Suppl 49) 13:190, 1978 (Abst)
64. Modlin, I.M., Christofides, N.D., Fitzpatrick, M.L., and Bloom S.R. : Effect of motilin on gastric emptying of solids in man. Scand. J. Gastroent. (Suppl 49), 13 :125, 1978 (Abst)
65. Besterman, H.S., Cook, G.C., Sarson, D.L., Christofides N.D. and Bloom, S.R. : Gut hormone profile in post-infective tropical malabsorption. Gut, 19:438, 1978 (Abst)
66. Besterman, H.S., Bloom, S.R., Christofides, N.D., Mallinson, C.N., Pera, A., and Modigliani, R. : Gut, 19:988, 1978 (Abst)
67. Besterman, H.S. Bloom, S.R., Adrian, T.E., Christofides N.D., Sarson, D.L., Mallinson, C.N., Pera, A., and Modigliani, R. : Gut hormone profile after gut resection. Gut, 19:972, 1978 (Abst)
68. Lucas, A., Christofides, N.D., Adrian, T.E., Bloom, S.R. and Aynsley-Green, A. : Foetal distress, meconium and motilin. Lancet, I:718, 1979 (Letter)

69. Bloom, S.R., Besterman, H.S., Adrian, T.E., Christofides, N.D., Sarson, D.L., Mallinson, C.N., Pera, A., and Modigliani, R. : Gut hormone profile following resection of large and small bowel. *Gastroenterology*, 76:1101, 1979 (Abst)
70. Bloom, S.R., Besterman, H.S., Welsby, P.D., Christofides, N.D., and Sarson, D.L. : Gut hormones in acute diarrhoea. *Gastroenterology*, 76:1102, 1979 (Abst)
71. Bloom, S.R., Christofides, N.D., Bryant, M.G., Buchan, A.M.J. and Polak, J.M. : One motilin or several? *Gastroenterology*, 76:1102, 1979 (Abst)
72. Bloom, S.R., Lucas, A., Christofides, N.D., Adrian T.E., and Aynsley-Green, A. : Motilin, aspects of its developmental physiology. *Gastroenterology*, 76:1103, 1979 (Abst)
73. Greenberg, G.R., Wolman, S., Christofides, N.D., Bloom, S.R., and Jeejeebhoy, K.N. : Gut hormone release after total parenteral nutrition in man. *Gastroenterology*, 76:1144, 1979 (Abst)
74. Reverdin, N., Hutton, M., Ling, A., Wingate, D.L., Ritchie, H.D., Christofides, N.D., Adrian, T.E. and Bloom, S.R. : The motor response to food : vagus-dependent or independent? *Gastroenterology*, 76:1125, 1979 (Abst)
75. Panayiotides, T., Christofides, N.D., Bjornsson, O.G., Bloom, S.R., and Chadwick, V.S. : Elemental diets, pancreaticobiliary secretions and gastrointestinal hormones. *Gut*, 20:450, 1979 (Abst)
76. Besterman, H.S., Welsby, P.D., Christofides, N.D., Sarson, D.L. and Bloom, S.R. : Gut hormones in acute diarrhoea. *Gut* 20:455, 1979 (Abst)
77. Rennie, J.A., Christofides, N.D., Bloom, S.R., Johnson, A.G. : Stimulation of human colonic activity by motilin. *Gut*, 10:912, 1979 (Abst)

78. Bloom, S.R., Chatei, M.A., Christofides, N.D., Adrian T.E., Blackburn, A.M., Lezoche, P., Basso, N., Carlei, F., and Speranzo, F. : Release of neurotensin, enteroglucagon, motilin and pancreatic polypeptide by bombesin in man. Gut, 10:913, 1979 (Abst)
79. Christofides, N.D., Long, R.G., Fitzpatrick M.L., and Bloom, S.R. : Motilin increases the rate of gastric emptying of glucose. Gut, 10:924, 1979 (Abst)
80. Bloom, S.R., Ghatei, M.A., Christofides, N.D., Blackburn, A.M., Adrian, T.E., Lezoche, E., Basso, N., Carlei, F., and Speranzo, F: Bombesin infusion in man, pharmacokinetics and effect on gastrointestinal and pancreatic hormonal peptides. J. Endocr., 83:51, 1979 (Abst)
81. Rennie, J.A., Christofides, N.D., Ellis, M.R., Mitchenere, P., Johnson, A.G. and Bloom, S.R. : Effect of motilin on human colonic activity. Clin. Sci. 58:12, 1980(Abst)
82. Barnes, A.J., Bottazzo, G.F., Doniach, D., Christofides, N.D., and Bloom, S.R. : Hormonal and metabolic studies in patients with circulating glucagon-cell and somatostatin- cell antibodies. Clin. Sci. 58:21, 1980 (Abst)
83. Bjornsson, O.G., Christofides, N.D., Fletcher, D., Bloom, S.R., and Chadwick, V.S. : Suppression of plasma levels of motilin during duodenal perfusion of sodium taurocholate in man. Gastroenterology, 78:1142, 1980 (Abst)
84. Blackburn, A.M., Fletcher, D.R., Christofides, N.D., Fitzpatrick, M.L., Adrian, T.E., and Bloom, S.R. : Neurotensin infusion in man and its effect on gastric function. Gastroenterology, 78:1142, 1980 (Abst)
85. Bloom, S.R., Ghatei, M.A., Bryant, M.G., Moody, A.J. and Christofides, N.D. : Molecular analysis of glucagon-one major form released after gut stimuli. Gastroenterology, 78:1980(Abst)

86. Long, R.G., Christofides, N.D., Fitzpatrick, M.L., McGregor, G.P., and Bloom, S.R. : Effects of motilin and somatostatin on gastric emptying and glucose tolerance. Gastroenterology, 78:1210, 1980 (Abst)
87. Rennie, J.A., Christofides, N.D., Mitchenere, P., Johnson, A.G. and Bloom, S.R. : Motilin and human colonic activity. Gastroenterology, 78:1243, 1980 (Abst)
88. Long, R.G., Christofides, N.D., Fitzpatrick, M.L., Mitchenere, P., and Bloom, S.R. : Somatostatin and motilin increase the rate of gastric emptying of glucose. Europ. J. Clin. Invest., 10:23, 1980 (Abst)
89. Rennie, J.A., Christofides, N.D., Mitchenere, P., Johnson, A.G., and Bloom, S.R. : Motilin and human colonic activity. Reg. Pep. (Suppl 1) 1:92, 1980 (Abst)
90. Lux, G., Femppel, J., Christofides, N.D., Lederer, P., Adrian, T.E., Rosch, W., Bloom, S.R. and Domschke, W. : Pancreatic polypeptide, motilin, and somatostatin levels during interdigestive motility in man. Reg. Pep (Suppl 1), 1:71, 1980 (Abst)
91. Bryant, M.G., Dawson, J., Christofides, N.D., Chatei M.A., Peeters, T.J., and Bloom, S.R. : Are the different molecular forms of enteroglucagon, motilin and gastrin stored in distinguishable intracellular granule types? Reg. Pep (Suppl 1) 1:15, 1980 (Abst)
92. Lux, G., Lederer, P., Christofides, N.D., Femppel, J., Adrian, T.E., Rosch, W., Bloom, S.R. and Domschke, W. : Hormonal regulation of interdigestive activity in man? Gut, 21:930 1980 (Abst)
93. Bryant, M.G., Ghatei, M.A., Moody, A. J., Christofides, N.D., Dawson, J., Peeters, T.J., and Bloom, S.R. : Gel permeation and subcellular fractionation analysis of the enteric and pancreatic glucagon hormones in tissue and plasma : can the differences be explained by post-translational enzymic processing? J. Endocrinol, 85:39, 1980 (Abst)

94. Adrian, T.E., Mitchenere, P., Sagor, G.R.,
Christofides, N.D. and Bloom, S.R. : Effect of motilin and
other gut hormones on gall bladder pressure. Reg. Pep
(suppl 1) 1:1, 1980 (Abst)

95. Long, R.G., Christofides, N.D., Fitzpatrick, M.L. and
Bloom, S.R. : Contrary effects of exogenous infusion of
somatostatin and motilin on glucose tolerance. Diabetologia,
19:295, 1980 (Abst)

96. Gibby, O.M., Greenwood, R.H., Mackman, J.M.,
Christofides, N.D., and Hales, C.N. : diazoxide inhibits
glucagon release in man. Diabetologia, 19:276, 1980 (Abst).

ACKNOWLEDGEMENTS

My thanks go in particular to Dr S.R. Bloom for his help and guidance during these studies. I am also very grateful to the following :

For their help in the various studies on the distribution, and release of motilin in both normal subjects and patients with gastrointestinal disease, Dr H.S. Besterman, Mr M.G. Bryant, Mr M.A. Ghatei, Mr D.L. Sarson, Dr J.M. Polak, Dr A. Lucas, Dr M.D.G. Gillmer, Dr A. Pera, Dr C.N. Mallinson, Dr A.M. Blackburn, Dr T.E. Adrian, Mr P. Mitchenere, Dr P. Mitznegg, Dr J. Dawson, Dr J.A. Rennie and Dr I.M. Schoon.

For their help in the infusion of motilin, Dr IM. Modlin and Dr R.G. Long. For the gastric emptying experiments Dr M.L. Fitzpatrick. For the upper intestinal motility measurements, Dr G. Lux, Dr W. Domschke and Dr G. Vantrappen.

I would also like to thank the many friends and colleagues who volunteered to undertake the experiments.

My special thanks go to my father, Mr Demetris D. Christofides and my sister, Mrs Christina Perdiou for typing this thesis.

CONTENTS

	<u>PAGE</u>
TITLE	1
DEDICATION	2
ABSTRACT	3
PUBLICATIONS/ACKNOWLEDGEMENTS	5/17
CONTENTS	18
LIST OF FIGURES	24
LIST OF TABLES	27
CHAPTER 1 : SHORT GENERAL HISTORICAL BACKGROUND	29
Introduction	29
Hormone isolation	30
Morphology	31
CHAPTER 2 : MOTILIN	32
Introduction	32
Isolation and Chemistry	33
Localisation	34
Actions of motilin	34
In vitro	34
In vivo	35
Pancreatic secretion	35
Protein synthesis	35
Electrical and mechanical activity	35
Gastrointestinal motility	36
Metabolism	36
CHAPTER 3 : RADIOIMMUNOASSAY OF MOTILIN	37
Introduction	37
History of radioimmunoassay	37
General principles	38
Radioactive label	40
Production of antibodies	41
Coupling methods	41
Glutaraldehyde	41
Carbodiimide	41
Diazonium salts	41

	<u>PAGE</u>
Carrier	41
Immunisation procedure	42
Separation Techniques	42
Problems in radioimmunoassay	43
Problems with the antibody	43
Antigen	44
Problems with the label	45
Assay sensitivity	45
Radioimmunoassay of Motilin	46
Production of antibodies	46
Carbodiimide method	46
Glutaraldehyde method	46
Immunisation	47
Collection of antisera	47
Antibody testing	47
Storage of antibodies	48
Characterisation of antisera	48
Scatchard plot	48
Preparation of the motilin tracer	49
Lactoperoxidase	49
Chloramine T	49
Testing of label	50
Specific activity - self-displacement) method)	50
Calculation of specific activity	50
Standard	50
Investigation of optimal assay conditions	51
Buffer and pH test	51
Charcoal test	51
Plasma motilin assay	52
Motilin free plasma	52
Preparation of gamma globulin	52
Preparation of antibody - sepharose) complex)	52
Washing of the antibody - sepharose) complex)	53
Stability of motilin in plasma and) whole blood)	54
Precision	54
Incubation time	55

	<u>PAGE</u>
Investigation of molecular forms of motilin) in tissue extracts)	57
Optimisation of extraction	57
Chromatography	58
Preparation of columns	58
RESULTS	59
Antibodies	59
Iodination profile	64
Optimal assay conditions	67
Buffer and pH	67
Charcoal test	67
Incubation times	67
Affinity immunoabsorption	67
Stability of motilin in plasma and) whole blood)	71
Quality controls/Extraction	73/75
Molecular forms of motilin	75
Human tissues	75
Porcine tissues	78
Canine tissues	79
Other Species	79
DISCUSSION	81
CHAPTER 4 : RELEASE OF MOTILIN	87
Introduction	87
Blood collection	8
Intraduodenal Stimulation	89
Acid	89
Alkali	89
Oral stimulation	89
Meal	89
Glucose	90
Fat	90
Protein	91
Water	91
Antral balloon distension	91

	<u>PAGE</u>
Intravenous stimulation	92
Nutrients	92
Insulin	92
Pancreatic polypeptide	92
Bombesin	93
Somatostatin	93
Atropine	93
Motilin during pregnancy and early life	93
Molecular forms in plasma	95
Chromatography	95
Subjects	95
RESULTS	97
Intraduodenal stimulation	97
Acid	97
Alkali	97
Oral stimulation	101
Meal	101
Glucose	101
Fat	101
Protein	101
Water load	112
Antral balloon distension	112
Intravenous stimulation	115
Nutrients	115
Insulin	115
Pancreatic polypeptide	115
Bombesin	126
Somatostatin	126
Atropine	126
Motilin during pregnancy and early life	126
Molecular forms in plasma	145
DISCUSSION	154
Release of motilin	154
Molecular forms	156
Motilin in pregnancy	157
Motilin in early life	157

	<u>PAGE</u>
CHAPTER 5 : PHYSIOLOGY OF MOTILIN	159
Introduction	159
Subjects	161
Gastric emptying of mixed meal	161
Gastric emptying method	161
Motilin infusion	162
Hormone radioimmunoassay	162
Calculations	163
Gastric emptying of glucose and fat	163
Subjects	163
In vitro validation	164
Gastric emptying method	164
Motilin infusion	164
Plasma chromatography	164
Motilin and the interdigestive	165
migrating motor complex in man	
Method	165
Analysis of pressure records	165
Subjects	166
Infusion of motilin	166
RESULTS	167
Gastric emptying of the mixed meal	167
Motilin levels	167
Gastric emptying rate	167
Pharmacokinetics	171
Effect of motilin on circulating)	
hormones and plasma glucose)	171
Gastric emptying of glucose and fat	171
Motilin levels in 'glucose' study	171
Gastric emptying of glucose	175
Effect of motilin on plasma)	
insulin and glucose	175
In vitro validation	179
Motilin levels in 'fat' study	179
Gastric emptying of fat	179
Plasma chromatography	179
Motilin and interdigestive migrating)	
motor complex in man	185

	<u>PAGE</u>
DISCUSSION	191
Gastric emptying	191
Pharmacokinetics	193
Motilin and interdigestive) migrating motor complex in man)	194
CHAPTER 6 : MOTILIN AND GASTROINTESTINAL PATHOLOGY	197
Introduction	197
Methods	197
Standard test breakfast	197
Patients	197
Infective diarrhoea	199
Tropical malabsorption	199
Crohn's disease	199
Ulcerative colitis	201
Diverticular disease	201
Irritable bowel syndrome	201
Short bowel syndrome	201
Controls	202
RESULTS	202
'Infective' diarrhoea	202
Tropical malabsorption	202
Crohn's disease	207
Ulcerative colitis	207
Diverticular disease	207
Irritable bowel syndrome	214
Short bowel syndrome	214
DISCUSSION	223
CHAPTER 7 : CONCLUSIONS	226
APPENDIX 1	230
RECIPES FOR BUFFERS	230
APPENDIX 2	231
CHEMICALS AND EQUIPMENT	231
REFERENCES	234

LIST OF FIGURES

<u>Number</u>		<u>Page</u>
301	Motilin conjugation profile	61
302	Motilin and motilin fragments dose-response curves (with antibodies M1 and GP 71)	62
303	Motilin and motilin fragments dose-response curves (with antibody M25)	63
304	Motilin iodination profile	66
305	Buffer test - Antibody M1	68
306	Charcoal test - Antibodies M1, GP71 and M25	69
307	Stability of motilin in plasma and whole blood	72
308	Molecular forms of motilin in human intestine	77
309	Molecular forms of motilin in porcine intestine	80
310	Motilin standard curves, in buffer and plasma (antibody M26) (example of 'non-specific' protein interference)	84
401	Motilin release after intraduodenal acid	98
402	Effect of intraduodenal alkalinisation on motilin	100
403	Motilin release following ingestion of a meal	102
404	Postprandial release of motilin in relation to the interdigestive myoelectric migrating complex	103
405	Motilin release following oral glucose	109
406	Motilin release following oral fat (cream)	110
407	Motilin release following oral protein (steamed cod)	111
408	Motilin release following an oral water load	113
409	Motilin release following antral balloon distension	114
410	Motilin release following intravenous nutrients	116
411	Effect of intravenous arginine on motilin	118
412	Plasma motilin following insulin induced hypoglycaemia	120
413	Plasma motilin during PP infusion	123
414	Plasma motilin during bombesin infusion	129
415	Plasma motilin during somatostatin infusion	130
416	Plasma motilin following atropinisation	132
417	Plasma motilin in normal weight pregnant women	136
418	Plasma motilin in obese pregnant women	138
419	Plasma motilin in preterm and term infants	143
420	Basal plasma motilin in fed and unfed pre-term infants (6th day of life)	144

<u>Number</u>		<u>Page</u>
421	Molecular forms of motilin in plasma following six stimuli	146
422	Molecular forms of motilin in plasma (antibody M1 and M25)	149
423	Correlation between peak II motilin and 'total' Plasma motilin (the latter measured with antibody M25)	150
424	Correlation between peak I motilin and the difference between plasma motilin values, measured by antibody M1 and M25	151
425	Log transformation of peak I motilin	152
426	Log transformation of peak II motilin	153
501	Plasma motilin during the gastric emptying of mixed meal	168
502	Effect of motilin on the gastric emptying of the mixed meal	169
503	Effect of motilin on individual half-emptying times of the mixed meal	170
504	Plasma motilin half-life following infusion	172
505	Plasma motilin during the gastric emptying of glucose (TC-99M)	174
506	Effect of motilin on the gastric emptying of glucose	176
507	Plasma Insulin and glucose during motilin infusion	178
508	Plasma motilin during the gastric emptying of cream (TC-99M)	180
509	Effect of motilin on the gastric emptying of cream	181
510	Molecular forms of motilin in plasma following ingestion of cream	182
511	A typical interdigestive migrating complex (donated by Professor G. Vantrappen)	186
512	Plasma motilin levels during an interdigestive migrating complex	187
601	Plasma motilin levels following breakfast in 'infective diarrhoea'	203
602	Plasma motilin levels following breakfast in Tropical malabsorption	205
603	Plasma motilin levels following breakfast in Crohn's disease	208

<u>Number</u>		<u>Page</u>
604	Plasma motilin levels following breakfast in ulcerative colitis	210
605	Plasma motilin levels following breakfast in diverticular disease	212
606	Plasma motilin levels following breakfast in Irritable Bowel Syndrome and diarrhoea	215
607	Plasma motilin levels following breakfast in Irritable Bowel Syndrome and constipation	217
608	Postprandial plasma motilin following partial ileal resection	219
609	Postprandial plasma motilin following partial colectomy	221

LIST OF TABLES

<u>Number</u>		<u>Page</u>
301	RIA principle	39
302	Stability of motilin	56
303	Motilin iodination test	65
304	Incubation time - motilin assay	70
305	Quality control - motilin assay	74
306	Distribution of motilin in human intestine	76
307	Distribution of motilin in canine intestine	79
401	Release of motilin after intraduodenal acid	99
402	Release of motilin following ingestion of meal	104
403	Plasma motilin after oral glucose	105
404	Plasma motilin after oral fat (cream)	107
405	Plasma motilin after oral protein (steamed cod)	108
406	Plasma motilin following intravenous nutrients	117
407	Plasma motilin following intravenous arginine	119
408	Plasma motilin during insulin - induced hypoglycaemia	121
409	Plasma motilin during insulin - induced hypoglycaemia (gastric acid drained)	122
410	Plasma motilin during 'low dose' PP infusion	124
411	Plasma motilin during 'high dose' PP infusion	125
412	Plasma motilin during 'low dose' bombesin infusion	127
413	Plasma motilin during 'high dose' bombesin infusion	128
414	Plasma motilin after atropinisation	131
415	Plasma motilin after glucose and meal in normal weight pregnant women (16 weeks of pregnancy)	133
416	Plasma motilin after glucose and meal in normal weight pregnant women (36 weeks of pregnancy)	134
417	Plasma motilin after glucose and meal in normal weight women (1 week postpartum)	135
418	Plasma motilin after glucose and meal in obese pregnant women (16 weeks of pregnancy)	139
419	Plasma motilin after glucose and meal in obese pregnant women (36 weeks of pregnancy)	140
420	Plasma motilin after glucose and meal in obese women (1 week postpartum)	141
421	Motilin immunoreactivity in 26 plasmas after gel chromatography (antibodies M1 and M25)	148

<u>Number</u>		<u>Page</u>
501	Effect of motilin on fasting and postprandial gastrointestinal hormones and glucose	173
502	Molecular forms of plasma motilin following ingestion of cream with a concomitant infusion of saline	183
503	Molecular forms of plasma motilin following ingestion of cream with a concomitant infusion of porcine motilin	184
504	Plasma motilin levels during an interdigestive myoelectric migrating complex	188
505	Motilin infusion and initiation of interdigestive myoelectric migrating complex	189
506	Characteristics of spontaneous and motilin - induced interdigestive myoelectric migrating complexes	190
601	Clinical details of all patients studied	198
602	Clinical details of patients with Tropical Malabsorption	200
603	Plasma motilin levels in 'infective diarrhoea'	204
604	Plasma motilin levels in Tropical Malabsorption	206
605	Plasma motilin levels in Crohn's disease	209
606	Plasma motilin levels in ulcerative colitis	211
607	Plasma motilin levels in diverticular disease	213
608	Plasma motilin levels in the Irritable Bowel Syndrome with diarrhoea	216
609	Plasma motilin levels in the Irritable Bowel Syndrome with constipation	218
610	Plasma motilin levels after partial ileal resection	220
611	Plasma motilin levels after partial colectomy	222

CHAPTER 1

SHORT GENERAL HISTORICAL BACKGROUND

Introduction

The first clear cut and successful experiment bearing on an endocrine gland was reported in 1849 by AA Berthold (1,2). He showed that castrated animals carrying testicular grafts, devoid of original nervous connections, exhibited normal sexual behaviour. He concluded that the testes released something into the blood that maintains male behaviour and the secondary sex characters.

In 1855 Claude Bernard (3) showed by chemical methods that the liver could release sugar directly into the blood and referred to this function as 'internal secretion'.

In 1889, Brown-Sequard, a French physician, aroused the interest of scientific world by attempting self-rejuvenation, at the age of 72, by taking subcutaneous injections of aqueous extracts of dog testes.

Furthermore the year 1889 was of prominence in endocrinology because of the report by von Mering and Minkowski (4) on the production of diabetes mellitus in the dog through surgical removal of the pancreas, implying the importance of this organ in the control of glucose homeostasis.

The real science of endocrinology was probably born through the experiments of Bayliss and Starling (5). Their work showed the existence and manner of action of the hormone secretin. This hormone is released from cells in the duodenal mucosa when acidified food enters from the stomach; it is conveyed by the circulation to the pancreas, where it stimulates the rapid discharge of pancreatic juice through the pancreatic duct. Although it had been recognised before this time that a variety of endocrine glands exert influences on the body, this discovery was epoch-making because it proved unequivocally, for the first time that chemical integration could occur without

assistance from the nervous system. It clearly confirmed the idea that special glands elaborate chemical agents, which are freed into the blood and exert regulatory effects upon distant target organs and tissues. Starling used the word "hormone" (Greek 'hormon', exciting, setting in motion) when referring to secretin, in 1905 (6).

The new subject of endocrinology was relatively easy to study. Removal of the appropriate gland gave the deficiency syndrome and injection of the glandular extract produced symptoms of excess. However, progress in defining the role of digestive hormones was slow. In the gut the endocrine cells though very numerous, were dispersed and not gathered into compact, manipulable glands. The gut is thus an example of the "diffuse endocrine system" which is certainly larger and may be more important than the traditional glandular endocrine system. It is however, far more difficult to study and it was not until 1959 that the first hormone, secretin (7), was finally isolated.

Hormone Isolation

In the isolation of gastrointestinal hormones there are two major obstacles to be overcome. The hormones occur in intestinal tissue in low concentrations when compared to hormones in discrete endocrine glands specifically developed for hormone biosynthesis or storage, and they are easily destroyed by the proteolytic enzymes of the intestinal tract. One of the major breakthroughs in the isolation of gut hormones was the observation by Bayliss and Starling of the thermostability of secretin (5). This was later found to be the case for all the gut hormones so far isolated. Thus, one of the first steps that has been used in the extraction of gut hormones is boiling the acid or water extract, which inactivates all the proteolytic enzymes, without affecting the nature of these hormonal peptides. Nonetheless, the size and difficulty of the task is illustrated by the fact that 10,000 hog intestines were required to produce a few milligrams of pure secretin (7). Further improvement in peptide purification techniques, including, for example, affinity chromatography, isotachopheresis, and high-pressure liquid

chromatography have greatly improved the extraction yield. However, because of the considerable bulk of fresh tissue that is required to isolate even small amounts of peptide, the work has mostly been carried out in animals, and the amino acid sequence of some of the human hormones is still unknown and could differ significantly from the originally isolated bovine or porcine forms.

Peptides from natural sources are very difficult to obtain, but once the amino acid sequence has been isolated synthetic peptides can be produced.

Morphology

The endocrine cells of the gut are diffusely dispersed in the mucosa between the epithelial non-endocrine cells. They usually fail to take up conventional stains and thus appear in a histological section as clear elements. There are a number of histological methods to show endocrine granules, of which the most widely used are those of silver impregnation. These can be used for light and ultrastructural microscopy and can be very helpful in distinguishing the various types of secretory granules present. However, the most reliable and specific method for the study of gut endocrine morphology, are the various techniques of immunocytochemistry which accurately define the nature of the cell product, together with electron microscopy which allows the examination of the fine ultrastructural features of the endocrine cell such as microvilli and secretory granules.

CHAPTER 2

MOTILIN

Introduction

In 1935, Shay and Gershon-Cohen (8) showed that luminal perfusion of the duodenum of human subjects with a 1% sodium bicarbonate solution produced an acceleration in the emptying of water and barium meal. More recently it was shown by Brown et al (9) in the dog, that the instillation of either fresh pig pancreatic juice, or 0.3M tris buffer pH 9.0 resulted in increased motor activity from transplanted fundic pouches. It was suggested that the increased motor activity of the fundic pouches was due perhaps to the release of a stimulatory agent from the duodenal mucosa. Brown (10) subsequently demonstrated that crude duodenal extracts containing cholecystokinin-pancreozymin (Boots pancreozymin) injected intravenously produced, in addition to the well documented pancreatic and gallbladder effects, an increase of motor activity in fundic pouches. This period of increased motor activity was followed by inhibition of spontaneous motor activity. A purer preparation of CCK (Cecekin, Vitrum, Sweden) was shown (11,12) to produce only an inhibition of motor activity in similar pouches. These studies suggested that, in the further purification of Cecekin from crude duodenal extract, a gastric motor activity stimulant had been removed. Boots Pancreozymin was subsequently subjected to gel filtration using Sephadex G75 in an attempt to separate the fundic pouch motor stimulant from the inhibitor, by testing the elution fractions for motor activity. This resulted in the separation of the motor activity stimulating substance. Having established that a stimulatory factor existed, Brown and colleagues then attempted to isolate and purify this factor.

Isolation and Chemistry

Side fractions produced during the purification of secretin (13) on carboxymethyl cellulose were obtained from Mutt and Jorpes (Karolinska Institute, Stockholm) and bioassayed for motor activity. The fractions possessing stimulatory activity were refractionated on CM-cellulose and the eluates (fractions 12-40) were further fractionated on sephadex G-25. The fractions which contained stimulatory activity were then subjected to a further fractionation step on DEAE-cellulose. Amino acid analyses revealed that the N-terminal amino acid was phenylalanine. Dansylation also confirmed the presence of tyrosine and lysine in the molecule. Digestion of the molecule with carboxypeptidase A revealed that the C-terminal residues were glycine and glutamine, the latter occupying the C-terminal position. Motilin is a 22 amino acid straight chained peptide with a molecular weight of 2700 daltons (14-16). The full sequence is as follows :-

Phe	val	pro	ile	phe	thr	tyr	gly	glu	leu	gln	arg	met	gln
1	2	3	4	5	6	7	8	9	10	11	12	13	14
glu	lys	glu	arg	asn	lys	gly	gln						
15	16	17	18	19	20	21	22						

Shortly after the publication of this sequence, the biologically active methionine-substituted peptide (13 Norleucine) motilin was synthesized by Wunsch (17). Subsequent successful syntheses have been achieved by Yajima (18) and Yanaihara (19). Structure-activity studies (20,21), using smooth muscles from stomach, duodenum and colon, revealed that the N-terminal region to be important for biological activity. Removal of the corresponding C-terminal amino-acids did not significantly diminish the biological activity. Also substitution of methionine (amino-acid 13) with either norleucine or leucine did not suppress the biological activity.

Localisation

The distribution of motilin in the human alimentary tract, assessed by radioimmunoassay (22), revealed that high concentrations were found in the human duodenum and jejunum and small amounts in the gastric antrum. Immunohistochemical studies (23-25) have shown it to be localised mainly in small, uniformly round, electron dense granules with a mean diameter of 160nm. These cells also contain numerous microfilaments. An additional type (much less numerous) of cell that stains predominantly by the N-terminal directed antisera, has the classical appearance of enterochromaffin cells with large, electron dense, polymorphic granules ranging from 200nm to 400nm in length with a cross-section varying from 200-280nm.

Actions of motilin

In vitro

Motilin has powerful effects on the smooth muscle of the gastric antrum, duodenum and colon (20,26). Smooth muscles from other organs (uterus, gallbladder etc) show no such response. There also appears to be a species difference regarding the actions of motilin; muscle strips from rabbit and man are highly sensitive whereas guinea pig and rat are refractory (27). The effect of motilin is about 50 times greater (in molar terms) than that of acetylcholine (28) and is not inhibited by atropine (anticholinergic), hexamethonium (ganglion blocker), tetrodotoxin (axonal blocker), or pheniramine (antihistaminic)(27). A direct effect of motilin on the muscle cell has been suggested. The contractile response to motilin is abolished by the Ca^{2+} antagonistic compound verapamil (27), thus suggesting that Ca^{2+} ions are involved in the action of motilin. As cyclic GMP is known to produce a rapid release of Ca^{2+} from microsomal fractions it is possible that cyclic GMP may also be involved in motilin-induced gastrointestinal smooth muscle contractions. It was reported recently that motilin-induced contraction of the duodenal muscle is associated with a concomitant increase of intracellular cyclic GMP (29).

In vivo

Pancreatic secretion

In dogs, infusion of 13-Nleu motilin, at doses of 0.6-37 pmol/Kg/h, was reported to increase pancreatic secretion (30) in a dose dependent fashion, the highest output being achieved at a dose of 18.5pmol/Kg/h. The highest bicarbonate output was found to be about 5% of the maximal response to secretin and the highest protein output about 35% of the maximal response to caerulein. When motilin was infused concomitantly with secretin, it was shown to inhibit the secretin induced bicarbonate secretion. In contrast, infusion of 13 Nleu motilin (37pmol/Kg/h) in humans (31) was found to have no effect on pancreatic, salivary or gastric secretion.

Protein synthesis

Infusion of 13-Nleu motilin in humans was reported to decrease significantly gastric mucosal protein synthesis as measured by the in vitro incorporation of C(14)-leu into protein.

Electrical and Mechanical Activity

According to the findings of Code and Marlett (33), in the fasting state a regular pattern of migrating myoelectrical (MMC) spike activity arises in the stomach or duodenum and then migrates in a caudal direction down the small intestine. After the MMC reaches the distal ileum another MMC begins in the stomach or duodenum. It has recently been shown that infusion of motilin (110-1000pmol/Kg/hr) resulted in a gastrointestinal motor response which resembled the naturally occurring MMC (34,35). Moreover it has also been reported, that elevation of plasma motilin (in the order of 100pmol/l) coincided with the initiation of a new MMC (in the dog)(36).

Gastrointestinal Motility

In 1976, Ruppin and colleagues reported (37) that infusion of 13-Nleu motilin (150 pmol/Kg/hr) accelerated the intestinal transit time of a barium sulphate meal, in human subjects. Paradoxically, these authors also reported (38) that infusion of graded doses of 13-Nleu motilin in three subjects resulted in a dose-dependent delay in the gastric emptying of a liquid test meal. The doses of motilin used were 36, 72 and 144 pmol/Kg/hr. In contrast to these findings Debas et al reported that infusion of natural porcine motilin (92-370 pmol/Kg/hr) in dogs, greatly accelerated the gastric emptying of liquids (39). Also Nakazawa found that bolus subcutaneous injections of motilin (370 pmol/Kg) in the rat accelerated the gastric emptying of saline (40).

Jennewein et al (41,42) and Meissner et al (43) indicated that motilin (37-110 pmol/Kg/hr) increased the basal tone of the lower oesophageal sphincter (LES) and induced phasic contractions of the LES. This effect of motilin was influenced by atropine or hexamethonium, suggesting the involvement of a neural mechanism. These observations have also been found to apply in humans (44).

Metabolism

The gastric motor stimulating activity of motilin was compared when it was infused into either the systemic circulation or the portal vein in conscious dogs. It was observed (36) that the biological activity in doses of 110-330 pmol/Kg/hr was only slightly inactivated by a single passage through the liver, suggesting that the liver is not a major site of motilin inactivation.

Shima et al (45) investigated plasma motilin levels in patients with chronic renal failure and found the concentrations to be elevated thus suggesting a possible role of the kidney in the clearance and inactivation of motilin.

CHAPTER 3

RADIOIMMUNOSSAY OF MOTILIN

Introduction

History of radioimmunoassay

In 1956, whilst investigating the metabolic fate of insulin in human subjects, Berson and coworkers (46) demonstrated that radioactively labelled insulin disappeared more slowly from the plasma of patients who had received long-term treatment with insulin, than normal subjects. Their results indicated that insulin antibodies had been acquired by these patients in response to the administration of insulin. Using ^{131}I - labelled insulin they were able to show that the binding of insulin to the antibody was reversible and that at a constant antibody concentration the ratio of bound insulin to free insulin was an inverse function of the insulin concentration.

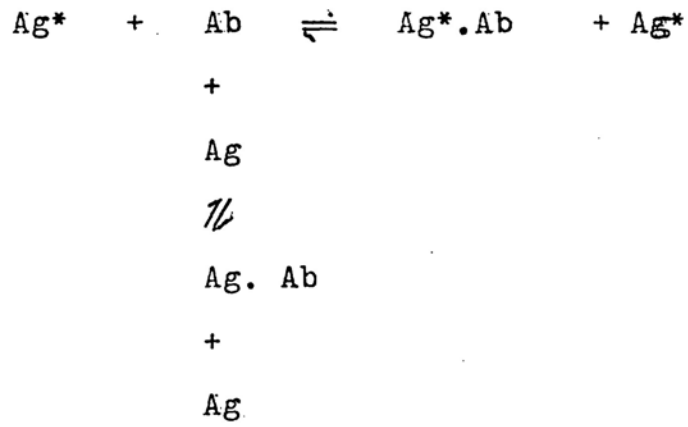
As a direct result of these observations, Berson and Yalow (47,48,49) developed the first radioimmunoassay system which enabled them to determine plasma insulin concentrations quantitatively by measuring the degree of competitive inhibition of binding of ^{131}I - insulin to human antisera. Similarly in 1959, (48) they measured the plasma insulin concentrations of healthy subjects using antibodies raised in guinea-pigs immunised with crystalline bovine insulin.

The development of radioimmunoassay techniques has proved to be the most significant milestone in the progress of endocrinology, because of its overall simplicity and potential for sensitivity. Radioimmunoassay has several distinct advantages over its preceding counterpart, the bioassay. It is usually more sensitive, tends to be more specific and the simplicity of the system permits the simultaneous estimation of many samples. Also the results obtained are often more precise and reproducible.

General Principles (50)

There are two basic requirements for the development of a radioimmunoassay system; a specific antibody and radioisotopically labelled hormone. The principle underlying the method is as follows : labelled hormone competes with unlabelled hormone for binding sites on the antibody. If unlabelled hormone is added in varying amounts to a system in which there are constant amounts of antiserum and labelled hormone, then the percentage of labelled hormone bound to the antibody will be inversely proportional to the amount of unlabelled hormone present (table 301).

As a result of this competitive inhibition, the percentage of labelled hormone bound to antibody is reduced progressively as the concentration of unlabelled hormone in known standard solutions or in unknown plasma samples is increased. By employing a technique whereby "free" labelled antigen may be separated from "antibody-bound" labelled antigen and then determining the amount of radiation present in each fraction in a radiation spectrophotometer, the concentration of endogenous hormone in plasma or tissue extracts may be determined by comparison of the observed "percent bound" with a curve relating percentage label bound to the concentration of hormone in known standard solutions.



Ag* is the radioactive ligand,
 Ag the cold ligand and
 Ab the antibody

Table 301

Principle of radioimmunoassay

Radioactive label

Radioimmunoassays employ a radioactive tracer molecule which is usually covalently linked to the antigen. For a successful radioimmunoassay the quality of the radioactively-labelled antigen is of prime importance. Ideally it should be homogeneous, of high specific activity, and should remain stable over a long period of time.

Several approaches exist for the introduction of radioactive isotope into a hormone. The approach that is most frequently used is the substitution of an isotope in a susceptible amino acid residue by chemical means (ie oxidation of tyrosine). A susceptible residue may have to be introduced chemically to allow such labelling if not naturally present. The radioactive marker may also be incorporated into a carrier molecule that is secondarily attached to the antigen (ie an iodinated succinamide ester residue).

The isotopes of iodine (I^{125} and I^{131}) are most frequently used as they are relatively easy to introduce into tyrosyl or histidyl residues (which are present in most gut hormones). ^{125}I is normally used in preference to ^{131}I because of its relatively longer half life (60 days as opposed to 8 days), which allows several months between the preparation of new batches of freshly labelled hormone, its greater isotopic abundance and the ease of counting the relatively low penetration gamma radiation emitted by this isotope in conventional gamma counters.

A variety of chemical methods exist for the introduction of iodine into hormones. Of these, perhaps the most widely adopted procedure involved the use of the mild oxidising agent *n*-chloro-*p*-methyl benzene sulfonamide, (chloramine T) (51). The reaction involves oxidation of ^{125}I iodide to free ^{125}I iodine and subsequent generation of the cation I^+ which is then incorporated into the aromatic ring of a tyrosyl residue in the peptide by electrophilic substitution. The reaction, which is very rapid, is terminated by addition of reducing agent, usually sodium metabisulphite which reduces unreacted chloramine T. Another oxidation technique used in the iodination of peptide

hormones involves the use of the enzyme lactoperoxidase obtained from unpasteurised bovine milk. In the presence of low concentrations of hydrogen peroxide, this enzyme oxidises free Na ^{125}I to allow incorporation into peptides (52). Once again it is the tyrosyl residue which is iodinated but by appropriate change of conditions it is possible to introduce the iodine into a histidyl residue.

Production of antibodies

Proteins in excess of 10,000 daltons usually promote a potent immunogenic response whereas polypeptides of lower molecular weight such as gastrointestinal hormones are often less antigenic. They can be rendered more antigenic when they are coupled as haptens.

Coupling methods

There are numerous agents that may be used for coupling haptens to proteins, of which the following three are commonly used :

1. Glutaraldehyde

Glutaraldehyde (53, 54) forms a bridge between amine groups (e.g N terminus or lysine) on both the protein and hapten.

2. Carbodiimide

Carbodiimides (55) condense free carboxyl and amino groups to form CO-NH bonds.

3. Diazonium salts

Diazonium salts bridge tyrosyl or histidyl residue of hapten and carrier.

Carrier

A large number of carrier proteins can be used (56, 57). Of these bovine serum albumin, because of its availability, immunogenicity, solubility and ease of coupling, tends to be the popular choice.

Immunisation procedure

The conjugate is injected in the form of an emulsion made up with Freund's (58) adjuvant (a mixture of n-hexadecane and Arlacel A). This permits slow release of the coupled hapten into the circulation and may serve to protect labile antigens against degradation (56). Freund's adjuvant alone will cause an inflammatory response which is thought to act as a stimulus to antibody formation. When made 'complete' by the addition of heat-killed mycobacterium, this response is further enhanced.

The emulsified conjugate can be administered in several ways, (56) including multiple intradermal injections, usually in the neck region or on foot-pads, intramuscularly, or subcutaneously (one into each groin and axilla). All these methods have been used successfully by a large number of investigators with high success rates.

Separation techniques

A wide variety of methods can be used to separate the free hormone from that which is bound to the antibody. The two methods that are most widely used are a) the double antibody (59, 60) and b) charcoal separation (61). The double antibody method exploits the immunological difference between label bound to antibody and free labelled peptide, and the charcoal method exploits their size differences. Double-antibody separation depends on the ability of the second antibody to bind the soluble antigen-antibody complex and cause precipitation by virtue of its now increased size. Charcoal used as a non-specific adsorbent is not only a simple method of separation but also allows rapid, inexpensive measurement of samples (62). Wood charcoal of particle size in the region of 60 μ is generally used. It is usually coated with dextran to define the pore size and so exclude large polypeptides and proteins.

Problems in radioimmunoassay

Although, as mentioned earlier, radioimmunoassay has several advantages over the bioassay, there are, however, a number of intrinsic problems. Firstly, the radioimmunoassay quantitates a specific amino acid sequence whilst the bioassay determines, what is often more physiologically relevant, the biological activity. Secondly, as the radioimmunoassay depends on the quantitative binding of the antigen to the antibody, is therefore very greatly influenced by any change in the molecular environment which causes alterations in the tertiary structure of the antibody or antigen. Thirdly, gastrointestinal hormones are not very antigenic, and circulate in low concentrations so that their measurement is nearly at the limit of assay sensitivity.

The problems that are normally encountered in radioimmunoassay revolve around the antibody and antigen. Ideally the antibody should be of high avidity, specific for the particular antigen, not susceptible to interference by such substances as urea or plasma proteins, and retain that avidity over a large range of pH changes, incubation times and charcoal concentrations.

Problems with the antibody

It is clear that any substance that interferes with antibody binding will give rise to erroneous results. Such substances can either be structurally related to the antigen or on the other hand could be quite different molecules. Related substances include prohormones abnormally produced, or degraded fragments of the hormone. A classical example of peptides with amino acid homologies are gastrin and cholecystokinin. Due to the common carboxyl-terminal pentapeptide sequence in these two hormones, many antibodies raised to one of these antigens react with both hormones by binding specifically to the antigenic regions of these hormones which are common for both peptides (63,64).

Another problem frequently encountered is that of non-specific protein interference of the antibody - antigen binding reaction. The error due to these effects can be reduced by the following means :-

1. Reducing the amount of the unknown sample in the assay tube. This results in a less sensitive assay and thus it is only of value when circulating concentrations are high and assay sensitivity is not a problem.
2. Extracting the hormone to be measured from plasma. This may introduce further errors and the efficiency of extraction may vary from sample to sample (65).
3. Including the same amount of a representative plasma, which is devoid of the hormone to be measured, in the standard curve. This hormone free plasma is usually prepared by one of three methods :-
 - a. hormone degradation by storage (eg the use of time - expired blood bank plasma)
 - b. adsorption to charcoal (61)
 - c. specific immunoabsorption (66) (affinity chromatography).

Time-expired blood bank plasma has the disadvantage of being diluted by significant volumes of anticoagulants and also materials released from damaged red cells, which may influence antibody binding. Some hormones, for example gastrin and pancreatic polypeptide, are remarkably stable and are not totally degraded in time-expired plasma. Charcoal adsorption has the disadvantage of removing all small peptides and other molecules besides, thus altering the molecular environment. The use of a hormone-specific immunoabsorbent therefore, is the method of choice (see later).

Antigen

The second important factor governing the success of a radioimmunoassay is the antigen. A highly purified antigen is required for both the preparation of the labelled tracer and for the reference standard. For high sensitivity assays a high specific activity tracer is of paramount importance.

Problems with the label

There are several special problems associated with the direct iodination of peptides, including :-

- a. Iodination of impurities. The contaminating peptide (s) may be more readily iodinated than the antigen in question.
- b. Lose of immunoreactivity. This may occur through the introduction of an iodine atom into a critical antigenic sequence of the peptide molecule. Oxidative damage(67), however, is perhaps the major factor which affects labelled peptide immunoreactivity.
- c. Decomposition during storage : Radiolabelled peptides deteriorate during storage. This may be due to the intrinsic instability of the peptide under storage conditions or may be due to the iodination reaction itself. The latter exposes the molecule to radiation damage (decay catastrophe) and may also produce other destabilising molecular changes during the initial oxidative iodination procedure.

Assay sensitivity

The assay sensitivity is the smallest change in hormone concentration that can be reliably detected by the assay. Two factors govern the sensitivity of an assay (68) - the steepness of the dose-response curve (eg standard curve), which is dependent upon antibody/antigen affinity, and the error (eg accuracy of a given value on the standard curve). Sensitivity is proportional to the gradient of the slope divided by the error. An increase in error will cause a reduction in the ability to reliably distinguish small hormonal changes. Error, which is random in direction and degree occurs in two main areas of radioimmunoassay, counting and handling technique (eg pipetting). The magnitude of the counting error is dependent upon the number of disintegrations counted. The more counts recorded, the lower the error $\left(\frac{100}{\sqrt{\text{Vcounts}}} \right)$.

The maximum sensitivity of an assay may be calculated as the smallest standard curve value measured, which is two standard deviations from the zero standard, ie 95 per cent confidence limit which approximates the two per cent slope from error calculations.

RADIOIMMUNOASSAY OF MOTILIN

Production of antibodies

Carbodiimide Method (55)

Two hundred and forty nmol (600 µg) of pure porcine motilin (kindly provided by Professor J.C. Brown, Department of Physiology, University of British Columbia, Canada) were conjugated with carbodiimide (CDI) to 90 nmol (6 mg) of Bovine Serum Albumin. The motilin and albumin were mixed together, dissolved in distilled water and then CDI was added. The solution was mixed gently and then incubated overnight at 4°C. Two fmol of iodinated motilin were also added to monitor the conjugation process (69).

Glutaraldehyde Method (53, 54)

Two hundred and forty nmol (600 µg) of porcine motilin and 90 nmol (6 mg) of bovine serum albumin were dissolved in 1 ml of 0.1 M phosphate buffer pH 7.5. Five hundred µl of 0.2 M glutaraldehyde were added slowly and then incubated overnight at 4°C. Again two fmol of iodinated motilin were also included to allow the assessment of the degree of conjugation obtained.

Two hundred µl of the two conjugation mixtures were then applied to the top of a 0.9x60 cm column containing 30 ml Sephadex gel, G-50 superfine. The columns were run at 6 ml per hour (0.01 M phosphate EDTA buffer, PH 7.4) at 4°C. Dextran blue and cytochrome C were included with the conjugation mixture and ran as internal column markers. Six minute fractions were collected and counted on an automatic multichannel gamma counter.

The conjugation mixtures were then made up to 6 ml with tap water. The Freund's adjuvant was made by mixing 10.2 ml of n-hexadecane and 1.8 ml Arlacel A. The adjuvant was made complete by addition of heat-killed mycobacterium. The emulsion was prepared by ejecting the separate conjugates, through a fine bore needle into the oil mixture. The mixture was repeatedly aspirated and re-ejected until an emulsion was formed. A simple test to show that the oil is in a continuous phase is lack of dispersion when a droplet of the emulsion is added to water.

Immunisation

Two ml of the emulsions were injected into New Zealand white rabbits (6 animals for each conjugate) via four 0.5 ml subcutaneous injections, one into each groin and axilla. The animals were given booster injections, 3 months after the primary immunisation (with half the amount) and monthly thereafter.

Collection of antisera

The rabbits were bled from the marginal ear vein, 7 and 10 days after the booster injections (peak antibody production is normally achieved within 10 days of boosting). The veins were dilated by means of a drop of xylene placed at the ear top and after a few minutes a small lateral incision was made into the vein, blood being allowed to drop freely into a container. After a suitable amount of blood was collected, the vein was clamped off and any remaining xylene washed from the ear.

Antibody testing

Blood was allowed to clot at room temperature for 1-2 hours. Red cells were then separated from the serum by centrifugation at 500g for 20 mins. The sera were then stored at -20°C, without any special preservative. The procedure used for preliminary antibody testing (for titre and slope was as follows):-

In total, eight tubes were used for each antibody. The first two tubes (a & b) contained 1 ml of buffer (0.01 phosphate EDTA buffer pH 7.4). Tubes (c & d) contained 900 µl whereas tubes, e, f, g, and h contained 800 µl of buffer. Five µl of neat antibody was added to tubes (a & b) and the tubes were thoroughly mixed. One hundred µl from these two tubes were then transferred to tubes (c & d) (a to c and from b to d), ten µl to tubes e & f and one µl to tubes g & h. Ten fmol of natural motilin (10 µl) was then added to tubes b, d, f and h. Two fmol of iodinated motilin was then added to all the tubes, and the tubes were incubated for 12-24 hrs at 4°C, after thorough mixing. They were then charcoaled (for conditions see later) and counted in a gamma counter. The antibodies with the steepest slopes were then tested fully, under optimal conditions.

Storage of antibodies

Antibodies that were shown to be good for radioimmunoassay (ie steepest slope and minimum non-specific interference) were diluted 1 part in 100 with non-immune rabbit serum and then stored in aliquots of 200 μ l at -20°C .

Antibodies which were of high titre but with a poor slope were stored undiluted at -20°C . These were used for affinity chromatography (see motilin free plasma).

Characterisation of antisera

The specificity of the antibodies was examined by serial addition of upto 2 nmol/tube of other gastrointestinal hormones including gastrin, cholecystokinin, secretin, vasoactive intestinal polypeptide (VIP), gastric inhibitory polypeptide (GIP), pancreatic polypeptide (PP), somatostatin, bombesin, substance P and neurotensin. The regional specificity of the antibodies was examined by serial addition of synthetic motilin fragments (Professor N. Yanaihara, Shizuoka, Japan). The peptides tested were Gln¹⁵ 1-22 motilin, the N-terminal 1-6-OMe and 1-17 aminoacid residues, the C-terminal fragments 7-22 and 12-22, the 7-17 motilin fragment, and 13-norleucine motilin (the last from Professor E. Wunsch, Munich, Germany).

Scatchard plot (70)

An effective method which can be used to estimate the binding affinity constant (K) of an antiserum is one based on the method of (70) Scatchard. In addition, the use of this plot to compare buffer and plasma assays is a useful index of the susceptibility of an antiserum to 'plasma effects'. Antisera which are not susceptible to these effects will give the same K value in plasma and buffer. The calculation is carried out as follows :-

- a. Calculation of the amount of hormone added as label to each tube (in femtomoles)
- b. Calculation of total hormone bound ie the amount of added labelled hormone plus the amount of cold hormone (standard) added, multiplied by the percentage bound to antibody, indicated by % radioactivity bound.

- c. Calculation of the dose response metameter B/F (bound radioactivity divided by free radioactivity) for each standard curve tube.
- d. Redrawing of the standard curve as Bound/Free on the vertical axis, against total hormone bound on the horizontal axis. Where the line intersects the vertical axis it gives $K \times N$, and where it intersects the horizontal axis it gives N, where N is in litres, and indicates the theoretical volume of antisera to bind one mole and K=Scatchard constant.

Preparation of the motilin tracer

The radioactive motilin label was prepared using both the lactoperoxidase and the chloramine T method.

Lactoperoxidase (52)

This was carried out at room temperature by serial addition of 50 μ l of 50 nmol/l acetate buffer pH 5.0, 2 millicuries iodine 125 and 1.5 nmol pure natural motilin. The reaction was initiated by addition of 10 μ g lactoperoxidase and 10 μ l of 0.86 mmol/l hydrogen peroxide and mixed by bubbling. After 15 mins 250 μ l of a 0.1 mol/l formic acid solution containing 1 mmol/l human serum albumin and 1 mmol/l potassium iodide was added and the mixture immediately applied to the top of a column containing about 30 ml of Sephadex G50 superfine gel. The product was eluted overnight at 4°C with 0.1 mol/l formic acid containing 5% human albumin.

Chloramine T (51)

Porcine motilin (1.5 nmol) was dissolved in 100 μ l of 0.2M phosphate buffer, (pH 7.4). Two millicuries of $\text{Na } ^{125}\text{I}$ was then added. The reaction was started by the addition of 180 nmol/l of Chloramine T, with bubbling. After 10-15 sec the reaction was terminated by the addition of 250 μ l of a solution that was made up by 0.5 ml human serum albumin, 10 μ l concentrated formic acid, 30 nmol potassium iodide and 0.5 μ mol sodium metabisulphite.

The iodinated mixture was applied to a Sephadex column (same conditions as above).

Testing of label

Each fraction containing radioactivity was tested in the assay for non specific adsorption to charcoal (blank) and for binding to an antibody present in excess (1/160 final dilution). This was carried out over a 1-2 hour incubation period at room temperature. The labelled fractions that appeared to contain the best motilin label (ie the lowest blank and the highest excess binding) were then reassayed under optimal condition.

Specific activity - self-displacement method (71)

The specific activity is defined as the radioactivity per mole of the antigen. The new SI unit of radioactivity is the Becquerel (1 disintegration per second = 0.27×10^{-10} Curies) and so the specific activity is expressed as Becquerel (Bq)/fmol. Pure iodine 125 has an activity of 81 Bq /fmol and thus any pure iodine 125 monoiodinated hormone will also have a specific activity of 81 Bq/fmol.

Calculation of specific activity

- a. Calculate the amount of radioactive hormone label added to the assay by reading off the standard curve a tube to which twice the usual amount of radioactive hormone has been added.
- b. In order to obtain disintegrations per second the efficiency with which the gamma counter performs must be known (this is approximately 33% for the gamma counter used for all these studies).
- c. Formula

$$\text{Specific activity (Bq/fmol)} = \frac{1}{\text{Counter Efficiency} \times \text{total tube count}} \times \text{Hormone per tube}$$

Standard

The porcine motilin standard was prepared gravimetrically. One hundred nmol (270 µg) was weighed, and then dissolved in freeze drying solution (see appendix 1).

Aliquots containing 1 pmol of motilin were transferred in glass vials and lyophilised in vacuo.

The lyophilised standards were kept at -20°C. New standards were prepared annually.

Investigation of optimal assay conditions

Several parameters were considered when optimising the motilin assay. These were :-

- a. Buffer and pH
- b. Amount of charcoal for separation
- c. Incubation time
- d. Plasma effects
- e. Stability of motilin
- f. Precision
- g. Investigation of molecular forms.

Buffer and pH

Three 'interlocked' standard curves were set up, one containing 0.05M acetate buffer pH5.0, another containing 0.06M Sorensens buffer pH7.0 and the last one containing 0.05M Sodium barbitone buffer (Veronal) pH 8.0 (for preparation of buffers, see Appendix 1). The standard curves were constructed by serial addition of the porcine motilin standard (0,1,2,3,5,10,15,20,25, 50,100 fmol/tube). The lyophilised standard containing 1pmol was reconstituted in 1 ml of buffer. The antibody to be tested, was added in a volume of 100 μ l and iodinated motilin (approx 2 fmol) also in 100 μ l. The final volume in every tube was adjusted to 800 μ l by addition of the corresponding buffer.

Charcoal

Separation of free from bound ligand was achieved using the charcoal technique. The quantity of charcoal required was investigated by noting the effect of increasing amounts (2 to 64 mg) on the assay blank (non-specific binding to charcoal) and zero binding. The charcoal suspension (containing 10% dextran relative to the charcoal concentration and 5% human plasma per 100ml of the mixture) was added as a 500 μ l slurry, the tubes thoroughly mixed and then centrifuged for 20 min. at 800-100g at 4°C. After centrifugation the supernatant was separated from the charcoal precipitate and both fractions counted on a gamma counter.

Plasma motilin assay

Motilin free plasma

The method used for preparing motilin free plasma was that of affinity immunosorption (66). This method employed the use of a high titre - low affinity antibody and was used for the specific removal of motilin from the plasma.

Preparation of gamma globulin

Two ml of saturated ammonium sulphate (diluted 1 to 4 after the saturation point was found) was added, dropwise with constant stirring, to six ml of antiserum (designated M4). The white precipitate of fibrinogen that was formed was removed after centrifugation for 10min at 800g. A further amount (1.5ml) of saturated ammonium sulphate was added, in the same manner, to six ml of the remaining supernatant. The solution was allowed to stand for 5 minutes allowing precipitation of the gamma globulin. This solution was then centrifuged for 10 mins at 800 g and the supernatant discarded. The precipitate was then washed with 10ml of saturated ammonium sulphate (diluted 1 to 2.5 after full saturation). The gamma globulin was dissolved in 1 ml of 100 mmol/l sodium bicarbonate 140 mmol/l sodium chloride buffer pH 8.0 and dialysed against the same buffer for 24 hours with one change of buffer.

Preparation of antibody - sepharose complex

Six grams of Cyanogen Bromide (CNBr) activated sepharose 4B beads were swollen and activated by placing the dry power in a scintered glass funnel (and washing with 3 litres of 3mmol/l hydrochloric acid followed by 1 litre of 100 mmol/l sodium bicarbonate/140 mmol/l sodium chloride pH8.0 buffer). This process lasted 15-20 minutes. The scintered glass funnel was affixed to a Buchner flask which was connected to a water driven vacuum pump. During the final wash with the bicarbonate/saline buffer the activated sepharose beads were suspended in approximately 20ml of this buffer. The dialysed gamma globulin preparation was diluted with the bicarbonate saline buffer to 20mls. This solution was immediately added to the activated sepharose. The mixture was allowed to react overnight at 4°C, mixing by gentle over and over rotation.

Any unreacted cyanogen bromide groups were deactivated by blocking with 500ml of 1mol/l glycine (or any other primary amine). The antibody/sepharose complex (now approximately 40ml) was placed in a flask containing 500ml of glycine. The mixture was gently mixed by rotation for 2 hours. After this period, the glycine solution was removed on a scintered funnel, again using a vacuum line. The preparation was then thoroughly washed and subsequently stored.

Washing of antibody - sepharose complex

Non-covalently bound antibodies were removed by extensive washing with alternate low and high pH buffers (100mmol/l sodium acetate pH4.0 and 100mmol/l sodium bicarbonate pH8.0) each containing 1 mol/l sodium chloride. One litre of each was used on each occasion. Four volumes of each buffer were washed through the complex. Finally, the beads were washed with 1 litre of sodium bicarbonate (100mmol/l) sodium chloride (140 mmol/l) buffer pH8.0 and then resuspended in 200ml of this buffer. Sodium azide was added as a bacteriostat at a concentration of 0.01% and the mixture stored at 4°C. The immunoabsorbent maintained activity for many months but required a further wash cycle prior to use, to remove the small quantities of antibody leached off during storage. The capacity of the motilin antibody covalently bound to sepharose beads was assessed by measuring the amount of added cold motilin which allowed 50% binding of motilin label (2fmol) when 1 ml of beads was incubated in 10ml 60mmol/l Sorensens buffer pH 7.0, for 1 hour at 4°C.

A large volume, usually about one litre, of pooled fresh heparinised plasma from healthy volunteers, was stripped using the motilin-specific affinity beads, the amount of beads added having been assessed by capacity testing. The plasma bead suspension was mixed by gentle rotation for two hours at 4°C and then the sepharose complex was removed by centrifugation. The stripping process was carried out three times and an aliquot of plasma was removed after each step to test the effectiveness of the preparation.

To test whether the measured basal plasma motilin levels were influenced by non specific assay error, all immunoreactive motilin

was removed from six unknown plasmas. One ml of each plasma was mixed for 1 hour with 20 μ l of the specific immunoabsorbent beads which were then removed again by centrifugation. To demonstrate that the motilin capacity of 20 μ l of beads added was more than adequate for complete removal of basal motilin 1 ml of the plasmas were also treated after additional motilin had been added to them to raise the motilin concentration by 150pmol/l. As a further control on any possible unknown effect of immunoabsorbent treatment 1 ml of each plasma was also incubated with sepharose beads to which gastrin antiserum had been previously conjugated by identical methodology to motilin beads.

Stability of motilin in plasma and whole blood

Gastrointestinal hormones tend to be unstable when kept, even for short times, in plasma or whole blood (46,48). For this reason the study of the stability of exogenous and endogenous motilin was undertaken. Blood samples from five healthy subjects were taken and aliquots were treated in the manner shown in Table 302.

The above experiment was also carried out following addition of Trasylol and also after addition of 50 fmol/ml of motilin (with and without 4000 KIU/10ml blood of Trasylol)(enzymatic inhibitor).

Precision

The interassay and intraassay variation of the motilin radioimmunoassay was monitored by including four 'quality controls' at the beginning and end of each assay run (in duplicate). The 'quality controls' were plasmas from healthy individuals lyophilised in 1 ml aliquots and kept at -20°C. The 'within assay' variation (intraassay) was calculated using the difference between replicates; 'between - assay' variation (interassay) was calculated from the average of replicates. The formula for calculating the coefficient of variation (CV) is $CV = \frac{SD}{\bar{X}} \times 100$ where SD is the standard deviation and $\bar{X}$ is the mean. "True" values were then assigned to each quality control plasma. This was estimated by averaging the values of 14 observations. Any assay which measured values falling outside two standard deviations (95% confidence limits) of this "true-value" was rejected.

Incubation time

Standard curves were set up, interlocked, and these were allowed to incubate for 1, 3 or 5 days at 4°C before they were charcoaled.

1. Plasmas frozen immediately
2. Plasmas frozen after 24 hours at room temperature
3. Plasmas frozen after 3 days at room temperature
4. Plasmas frozen after 10 days at room temperature
5. Plasmas frozen and thawed 5 times
6. Plasmas frozen and thawed 20 times
7. Whole blood kept for 3 hours at room temperature then spun and plasmas frozen
8. Whole blood kept for 24 hours at room temperature then spun and plasmas frozen.

Table 302

Stability of motilin in plasma and whole blood - different protocols employed.

Investigation of molecular forms
of motilin in tissue extracts

Optimisation of extraction

A study was carried out in order to establish optimal conditions for extracting motilin from gastrointestinal tissue. The efficiency of the extraction technique was studied using iodinated motilin tracer as the internal marker. Gastrointestinal tissues were boiled, and when cool 10 fmol of iodinated motilin was added. The tissues were then homogenised and extracted twice in water and twice in 100 mmol/l of formic acid. The amount of the motilin tracer extracted with each step was measured using a gamma counter. An alternative method also used, was to extract duodenal tissue until no more motilin was detectable in the extract. The motilin content after each extraction step was then measured by radioimmunoassay and the extraction recoveries calculated.

Human gastrointestinal tissue from the antrum (n=5), duodenum (n=5) and jejunum (n=5) was obtained fresh at surgery (department of Surgery, Hammersmith Hospital, London). Although thought not to be clinically involved in the various pathological processes, each piece of tissue was subjected to microscopical and histological examination. Only normal tissue was included in this study. Porcine tissue was obtained under halothane anaesthesia. The tissues were rapidly separated into wall and mucosa (carried out by the Dept of Histochemistry, Hammersmith Hospital, London) and were immediately frozen and stored at -20°C. Extraction was accomplished in the following manner: frozen mucosa was finely crushed and plunged into boiling water (5ml/g) held for 10 mins at 100°C, cooled, then homogenised in the same solution, centrifuged (at 800-1000g) for 10 mins and the sediments reextracted twice in two lots of 5 ml of 100mmol/l formic acid. The extracts were stored at -20°C until assay. Upper intestinal tissue from other species including dog, rabbit, rat, mouse and frog were also extracted in the same manner.

Chromatography

Tissue extracts were applied to the top of a 0.9x60 cm Pharmacia column, containing 30ml of gel, Sephadex G50 superfine. Columns were run at 6ml per hour at 4°C and were precalibrated with dextran blue, cytochrome C, sodium I¹²⁵ and porcine motilin. Dextran blue and cytochrome C were included with each sample run as internal markers. A pilot study carried out with three elution buffers (0.01M EDTA, 0.06M phosphate buffer pH 7.4, 0.2M ammonium bicarbonate pH 8.5, and 0.1M formic acid pH 2.8), showed that the chromatographic profiles of motilin immunoreactivity were identical. Thus the phosphate buffer, (as it was the same as the assay buffer), with addition of 2% human serum albumin was used thereafter. The eluted material was collected by means of an automatic fraction collector, calibrated at 6 min/fraction. The eluted fractions and also the neat material that was chromatographed were assayed for their motilin content and thus an estimate of the recovery was calculated. Three regional specific antibodies were used for these assays (see results). Several extracts were treated with 8M urea before loading on the column. Some of the fractions that contained motilin immunoreactivity were assayed in various dilutions, to test for parallelism. The elution positions were referred to by means of the coefficient of distribution (72) (K_{av}) = $(V_e - V_o) / (V_t - V_o)$, where V_o is the elution volume of the substance completely excluded from the gel (ie dextran blue), V_e is the elution volume of the substance studied and V_t is the total volume of the gel bed.

Preparation of the columns

Four grams Sephadex gel, G50 superfine, were pre-swollen in distilled water (overnight at 4°C). The water was then discarded and 10mmol EDTA, 60 mmol phosphate buffer pH 7.4 containing 2% human serum albumin was added. The gel was then gently mixed and poured onto the column. Care was taken so that no air bubbles were present (gently tapping the outside of the column). The gel was added, until the packed gel bed was about 10cm below the top of the column. The elution buffer was then pumped through the gel at 6ml/hour for 12 hrs. The tissue extracts were always centrifuged, in order to

remove any solid debris, and aliquots were then gently applied to the top of the gel bed. Care was taken so that the surface of the gel was not disturbed.

RESULTS

Antibodies

Figure 301 shows the chromatographic profile of the motilin label in the conjugation mixture, (the figure shows the results from a typical carbodiimide conjugation). The control, ie motilin label and bovine serum albumin, in the absence of the coupling agent, is depicted by broken lines. Conjugation of less than forty per cent was considered unacceptable. Under these circumstances more of the coupling agent was added and the mixture further incubated.

All the rabbits immunised with the carbodiimide conjugate produced antibodies. The final titre of these antibodies (that was sufficient to cause a 50% binding of the motilin label) ranged from 1/10,000 to 1/320,000. Preliminary testing with synthetic motilin fragments revealed that four antibodies were N-terminal specific whereas the other two were C-terminal specific. Of these antibodies only one was of sufficient affinity for use in radioimmunoassay (this antibody was designated as M1). Another antibody (M4) appeared to have a high titre (1/320,000 final) but a very shallow slope. This was used for affinity immunoabsorption.

Using the glutaraldehyde conjugation five of the six rabbits produced antibodies (three were C-terminal specific and two were N-terminal specific). Again only one (M25) looked promising for use in radioimmunoassay.

Figures 302 and 303 show the dose response curves of the various synthetic motilin fragments with three antibodies (M1, GP71 and M25). Antibody GP71 was raised in a guinea pig using porcine motilin conjugated by carbodiimide to bovine serum albumin (this antibody was kindly donated by Professor J.C. Brown, Department of Physiology, Vancouver, Canada).

The amount of natural porcine motilin required to halve (50%) the percentage binding (expressed as 100%) was 15fmol with M1, 38fmol with GP 71 and 16fmol with M25.

Substitution of the methionine residue with norleucine, at position 13 (not shown in the figures) resulted in full crossreaction with all antibodies. Substitution of glutamic acid (position 15) with the glutamine residue resulted in full crossreaction with M1, but there was a shift to the right in the dose-response curve with antibodies GP71 and M25. The calculated cross reactivity, with these antibodies, was only 40%. Partial crossreaction was also seen with the C-terminal fragments 7-22 (55%) and 12-22 (7%) with GP71 but not with M1. Fragment 7-22 exhibited full crossreaction with M25 whereas fragment 12-22 showed only a 40% crossreaction. The N-terminal fragment 1-17 exhibited an appreciable degree of crossreaction (57%) with M1, but not with GP71 or M25 (41%). Fragments 1-6 OMe and 7-17 did not crossreact with any of the antibodies. None of the other hormones appeared to crossreact with any of the antibodies. The affinity constants of antibodies M1, GP71 and M25 calculated by Scatchard plot analysis were $1.5 \pm 0.1 \times 10^{11}$ l/mol, $1.40 \pm 0.13 \times 10^{11}$ and $1.6 \pm 0.2 \times 10^{11}$ l/mol respectively. The affinity constants were not altered when they were set up in motilin - free plasma.

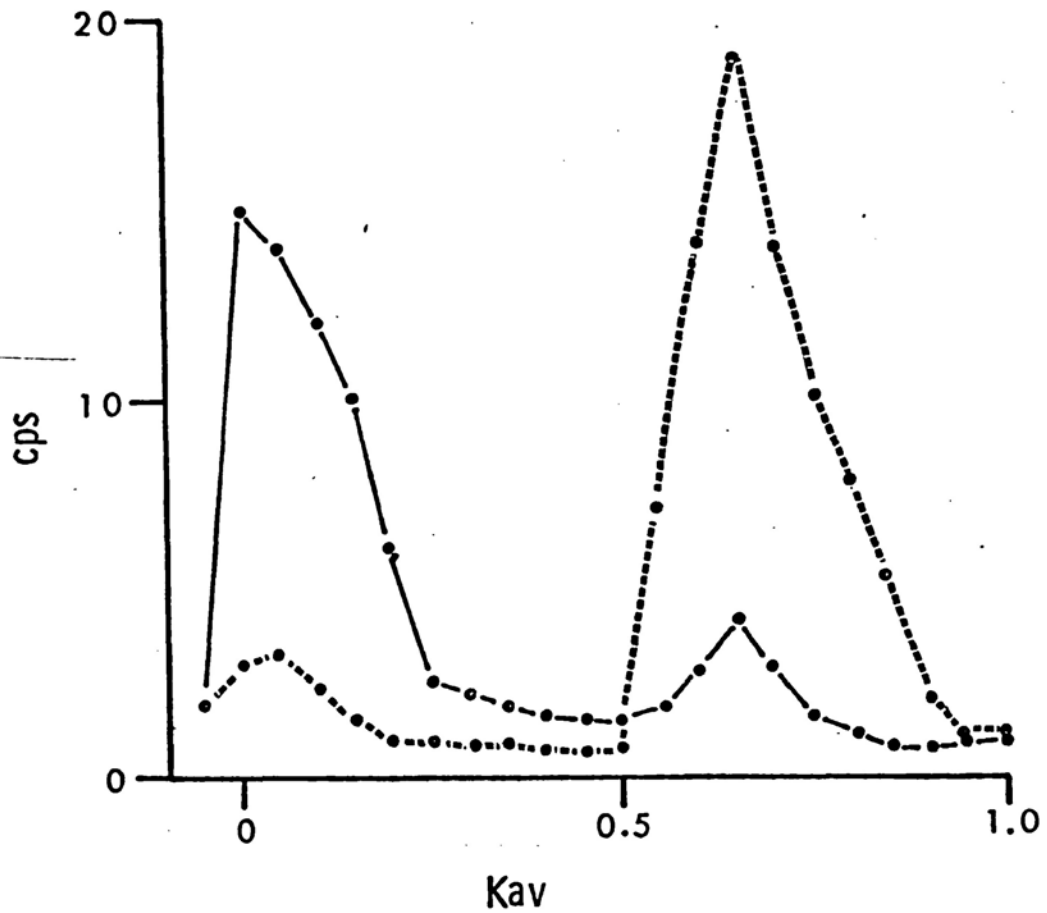


Figure 301

Chromatographic profile of motilin label in the conjugation mixture (solid line) and control (ie motilin label and BSA) (broken line).

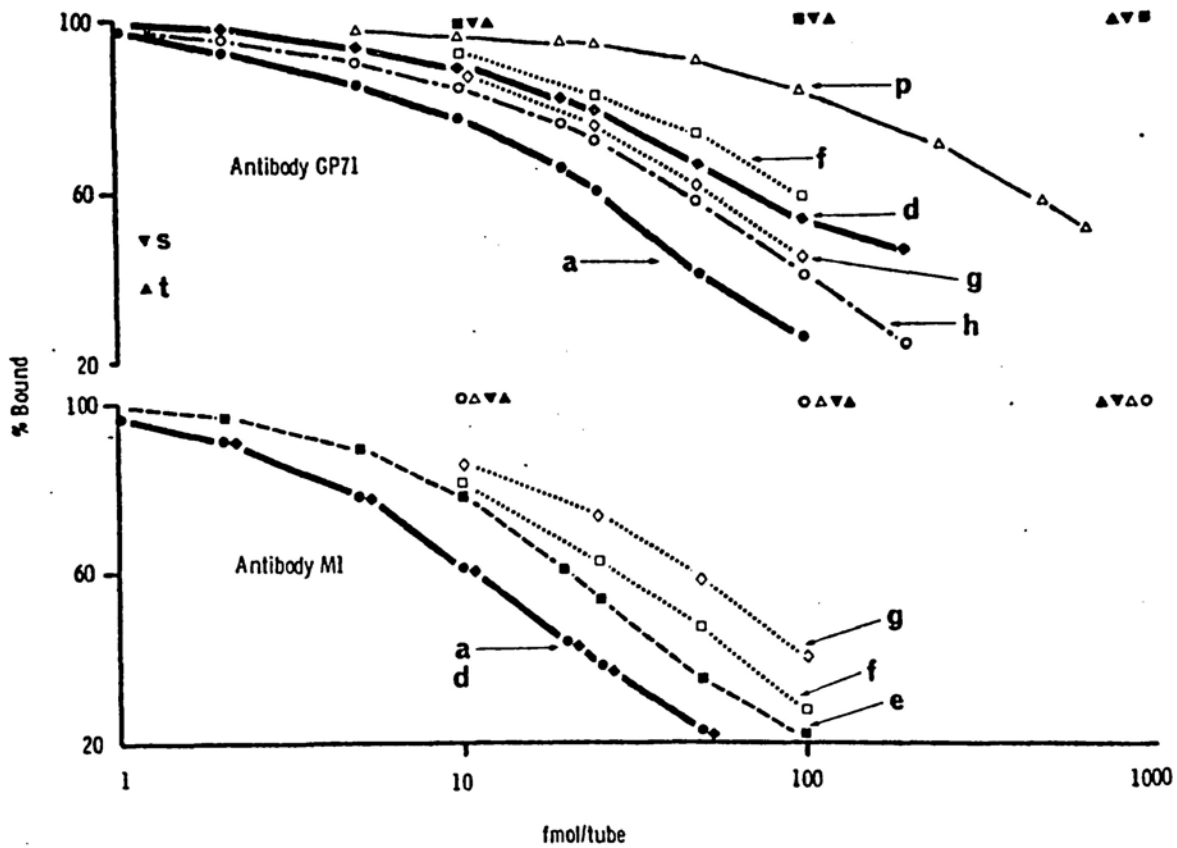


Figure 302

Dose-response curves of the various motilin fragments and analogues (and also human peak I and peak II motilin immunoreactivity) using antibody M1 (bottom panel) and GP71 (top panel). a:(porcine motilin), d:(Gln¹⁵-motilin), e:(1-17 motilin), f:(dilution curve of peak I motilin at 10,25,50 and 100 μ l), g:(dilution curve of peak II motilin at 10,25,50, and 100 μ l), h:(7-22 motilin), p:(12-22 mitilin), s:(1-6 OMe motilin), and t:(7-17 motilin).

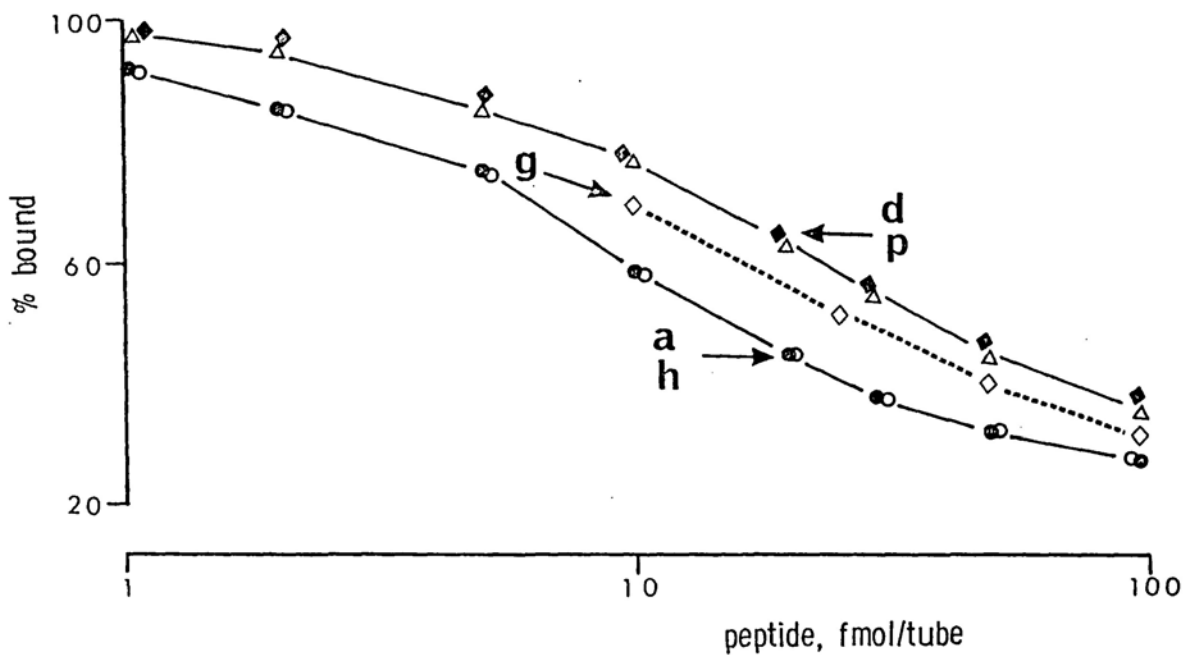


Figure 303

Dose-response curves of the various motilin fragments and analogues (and also human peak II motilin immunoreactivity) using antibody M25. a:(porcine motilin), d:(Gln¹⁵-motilin), g:(dilution curve of peak II motilin at 10,25,50 and 100 μ l), h:(7-22 motilin), p:(12-22 motilin). Motilin fragments 1-17, 7-17 and 1-6 did not crossreact significantly.

Iodination Profile

Figure 304 shows a typical motilin iodination profile. This particular profile resulted from a chloramine T oxidation. Similar profiles can also be obtained using the lactoperoxidase technique. The horizontal axis represents the various elution fractions (0.8ml fraction). The vertical axis shows the amount of gamma radiation (in counts per second, counted at a distance of 60 cm from the gamma counter) in each fraction.

As can be seen three radioactive peaks were observed. The three peaks eluted at fractions 20, 40 and 51 (peak fraction). Preliminary testing of the peaks (blank and excess, one hour incubation) suggested that the second peak contained I^{125} - motilin (see table 303).

The specific activity (on a subsequent test, under optimal conditions) on the descending side of the peak (ie fractions 41 and 42) was 63-76 Bq/fmol (1.7-2 nCi/fmol) whereas on the ascending part was below 40 Bq/fmol. Fractions 41 and 42 were thereafter diluted, and aliquots were taken and kept deep frozen.

The third peak appeared to be free Na I^{125} as tested by the starch iodide paper (in the presence of an oxidising agent (chloramine T), iodine turns starch iodide paper into a bluish colour.

<u>Fraction</u>	<u>Blank</u>	<u>Excess</u>
	% Binding	
20	80%	80%
39	4.9	89.6
40	4.7	90.3
41	4.6	91
42	4.1	90.3
51	62.4	67
'old' label	6.4	90.6

Table 303

Preliminary label test (using blank and excess at 1 hour incubation).

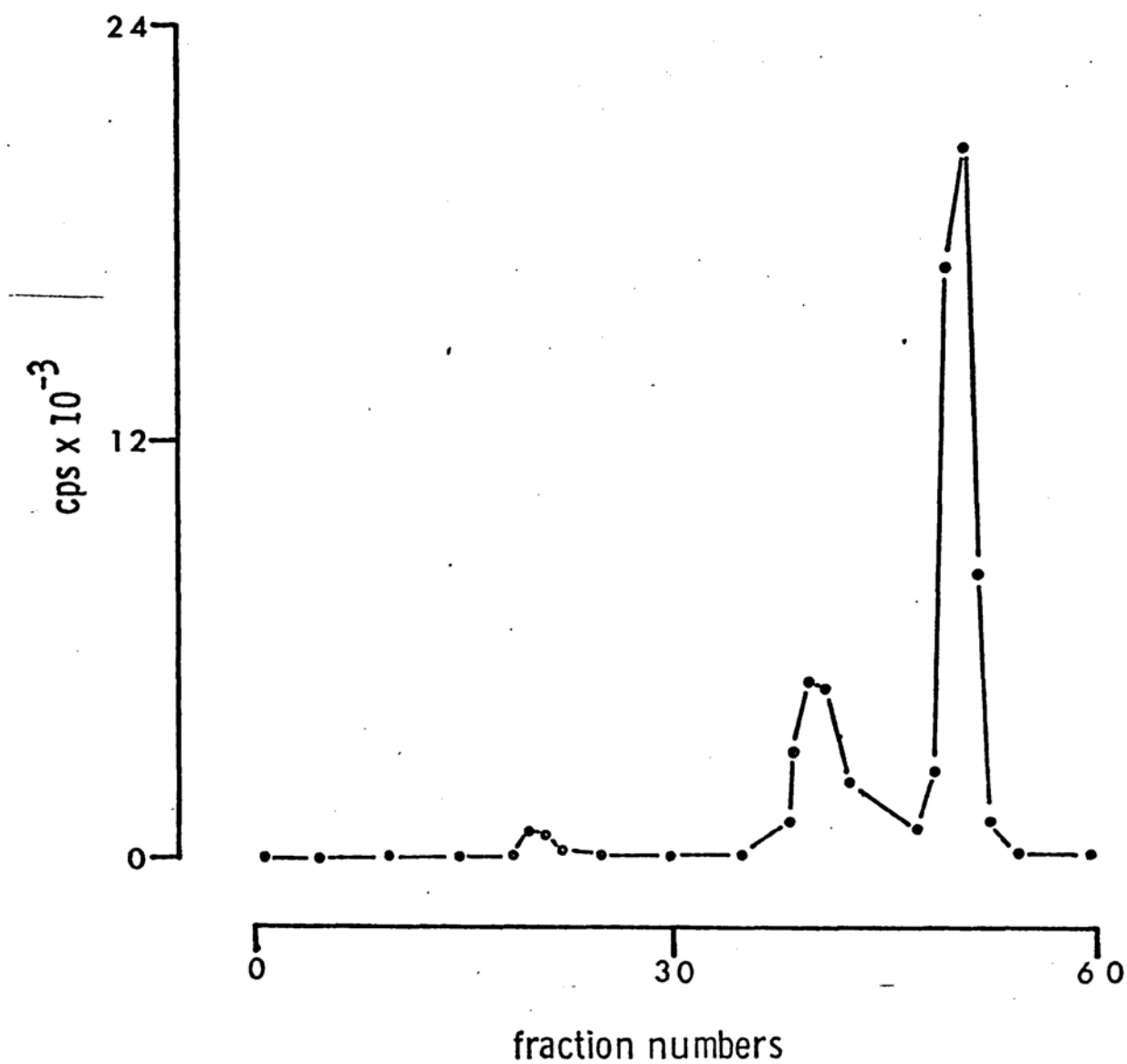


Figure 304

Motilin iodination profile (using a G-50 superfine column).

Optimal assay conditions

Buffer and pH

Figure 305 shows the effect of various buffers on the dose response curve (standard curve) with antibody M1. The zero binding appeared to be higher and the slope was much steeper in the Sorensens' buffer when compared to the other two buffers. Antibodies M25 and GP71 behaved in a similar fashion to M1.

Charcoal test

Figure 306 shows the effect of increasing amounts of charcoal on the assay blank and zero binding of antibodies M1, M25 and GP71. Thus the amount of charcoal used thereafter was 6mg/tube for all antibodies, both in buffer and plasma.

Incubation times

Table 304 shows the effect of various incubation times on the zero binding and slope with the three antibodies. Thus incubation times of three to five days were subsequently used throughout.

Affinity Immunoabsorption

The capacity of the motilin immunoabsorbent sepharose beads was 20 nmol/ml. The mean apparent motilin concentration in the six plasmas prior to treatment with the immunoabsorbent beads was 87 ± 14 pmol/l and after treatment 3 ± 8 pmol/l. After cold motilin was added to the plasmas the mean content was raised to 230 ± 16 pmol/l and bead treatment reduced this to 6 ± 8 pmol/l. Treatment with the immunoborbent gastrin beads did not significantly affect the motilin level. The blank values of the plasmas remained constant suggesting that no loss of antibody occurred.

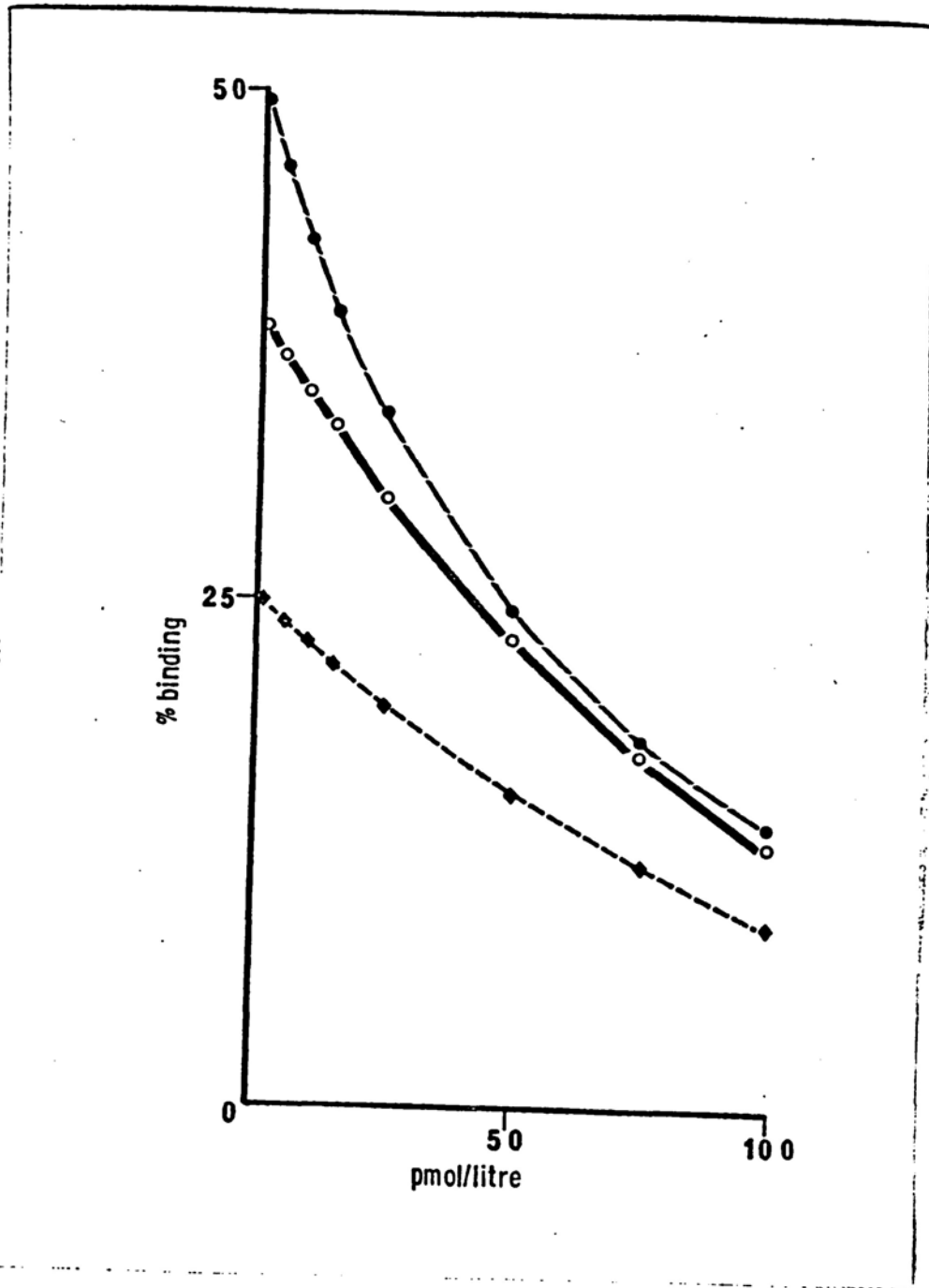


Figure 305

Dose-response curves, using antibody M1, in Sorensens buffer, pH 7.0 (solid dots), acetate buffer, pH 5.0 (open dots) and Veronal buffer pH 8.0 (solid squares).

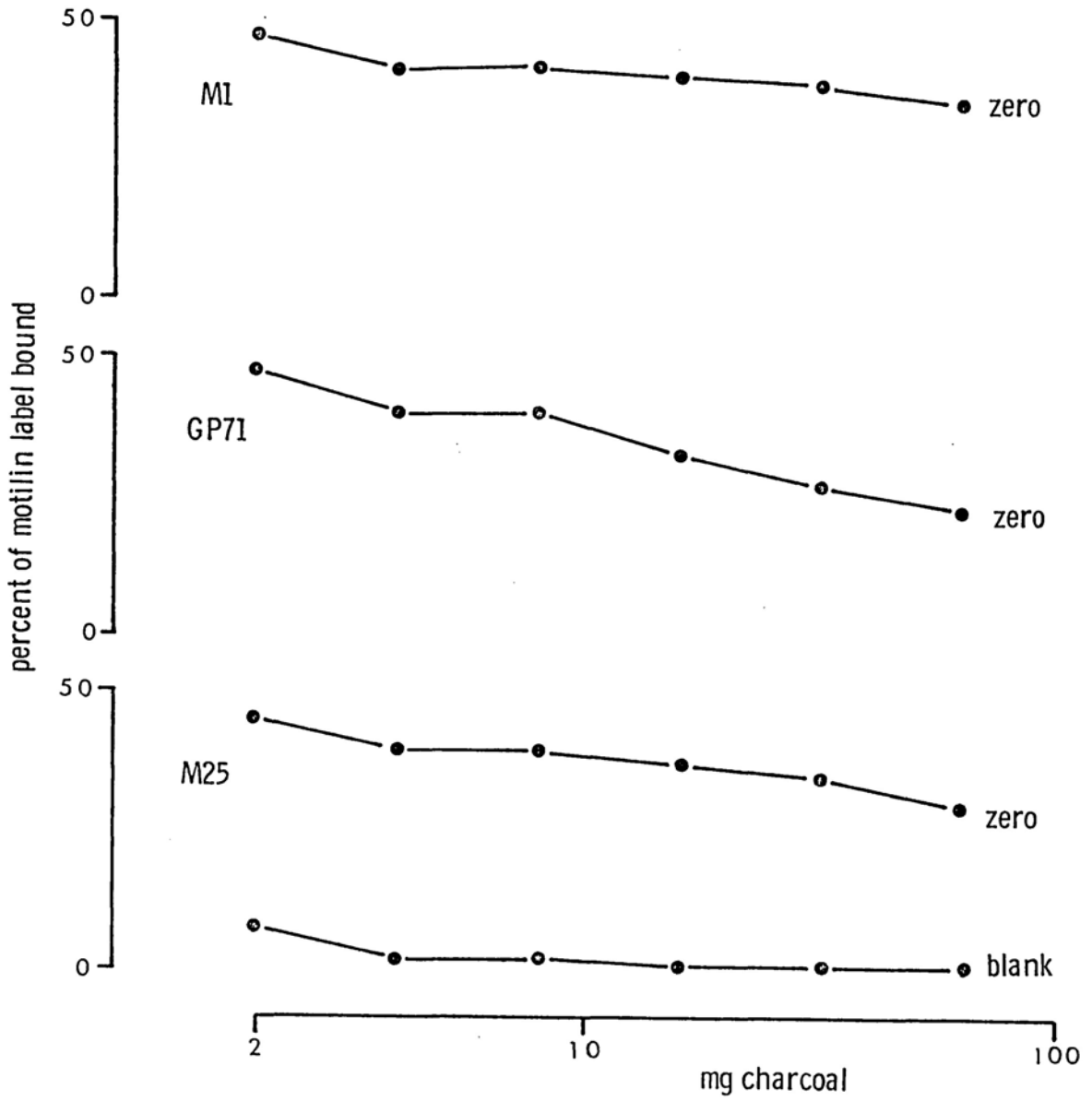


Figure 306

Charcoal Test. The effect of increasing amounts of charcoal on the blank and zero binding using antibody M1(top), GP71 (middle) and M25 (bottom).

Antibody M1

	1 day	3 days	5 days
Zero binding	39	53	54
2 Fmol/tube	36	46	44
5 Fmol/tube	32	38	37
10 Fmol/tube	26	30	28

Antibody M25

Zero binding	34	40	42
2 Fmol/tube	31	32	32
5 Fmol/Tube	27	26	25
10 Fmol/tube	23	22	21

Antibody GP71

Zero binding	31	45	47
2 Fmol/tube	27	39	39
5 Fmol/tube	23	33	31
10 Fmol/tube	19	26	25

Table 304

Effect of different incubation times on the zero binding (%) and sensitivity, using antibodies M1 (top), M25 (middle) and GP 71 (bottom)

Stability of motilin in plasma and whole blood

Figure 307 shows the degree of degradation of motilin (endogenous and/or exogenous) in plasma or whole blood, in the presence or absence of Trasylol. Motilin appeared to be relatively stable with only about 25% degraded after 3 days at room temperature. Incubation for 10 days resulted in a 50% degradation. There was no improvement with Trasylol. There was only a minor degradation when whole blood was kept at room temperature for 24 hours.

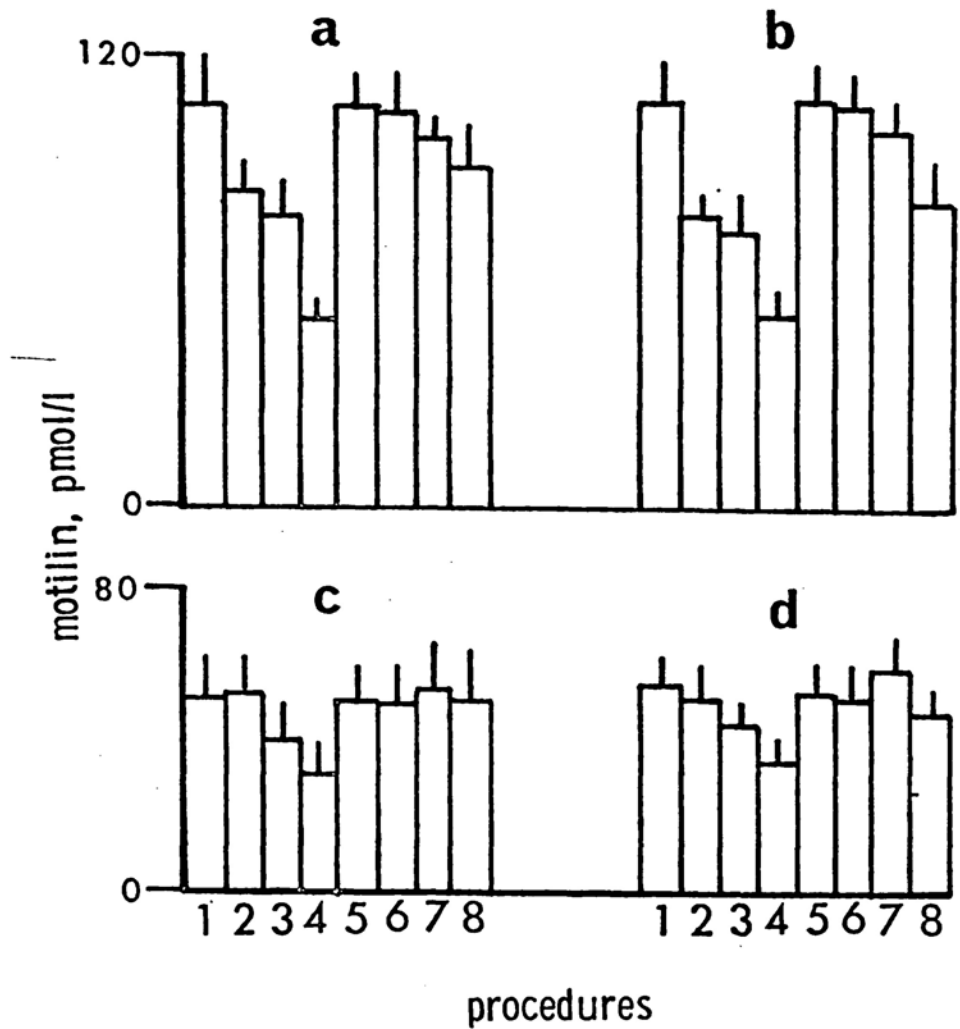


Figure 307

Plasma motilin immunoreactivity following the protocol depicted in table 302 (numericals denote the different procedures).

- a : (with the addition of 50 fmol/ml of motilin)
- b : (addition of motilin in the presence of Trasylol)
- c : (endogenous motilin)
- d : (endogenous motilin and Trasylol)

Quality controls

The 'true' values of 4 quality control plasmas (14 consecutive estimations) were 26, 42, 80 and 110 pmol/l. The intraassay variation for plasma 1 was 5% for plasma 2, 4%, for plasma 3, 4% and for plasma 4, 2.7% (table 305 shows the individual values for quality control plasma 4). The interassay variation in the 4 plasmas was 12%, 7.5% and 3.4% respectively.

Table motilin QC Plasma 4

Assays	R e p l i c a t e s				A v e r a g e		
	1st	2nd	3rd	4th	replicates		
					$\bar{x}$	SD	CV
1	110	108	105	107	107.5	2.08	1.9
2	115	125	112	108	115	7.2	6.3
3	120	122	115	115	118	3.55	3
4	104	110	106	110	107.5	3	2.8
5	105	111	110	108	108.5	2.64	2.4
6	108	108	112	110	109.5	1.9	1.7
7	106	110	108	111	108.8	2.2	2
8	106	106	110	108	107.5	1.9	1.8
9	108	118	110	112	112	4.3	3.8
10	106	106	109	110	107.8	2.06	1.9
11	116	120	114	116	116.5	2.52	2.2
12	113	110	109	116	112	3.16	2.8
13	104	108	109	107	107	2.16	2
14	102	110	106	109	106.8	3.59	3.4
$\bar{x}$					110.3		
SD					3.77		

Table 305

The mean intraassay variation of QC plasma 4 is 2.7%.
The inter-assay variation is 3.4%.

Extraction

The percentage sequential extraction recovery in water and acid (both with the addition of exogenous motilin and extraction of the endogenous motilin) was $71 \pm 4\%$ (1st water); $15 \pm 2\%$ (2nd water), $11 \pm 3\%$ (1st acid) and $3 \pm 1\%$ (2nd acid). No significant endogenous (or radioactive) motilin was detected after 4 extraction steps.

Molecular forms of motilin

Human tissues

Figure 308 shows a representative chromatographic profile from human antral mucosa, duodenal mucosa, and jejunal mucosa. Two immunoreactive motilin peaks were detected in each tissue by antibodies M1 and GP71. The first peak (peak I) eluted at a position suggesting a large molecular size substance ($K_{av}=0.16$), while the second peak (peak II) eluted at the same position as the natural porcine motilin standard ($K_{av}=0.67$). Antibody M25 appeared to detect only peak II. Urea pretreatment of the samples did not affect these profiles. Both peaks, when assayed in serial dilution (with antibodies M1 and GP71), exhibited parallelism (figure 302). Recovery of total motilin immunoreactivity during chromatography was always between 80 and 100%. No difference was seen in the profiles and relative proportions of the two immunoreactive peaks between water and acid tissue extracts.

Table 306 shows the amount of motilin immunoreactivity in peak I and II for all regions of the human gastrointestinal tract. More of the motilin immunoreactivity detected with M1 appeared to be the high molecular weight substance. There was no difference in the relative concentration of this material in the three regions studied (antrum, duodenum and jejunum). The amount of the first motilin peak ($K_{av}=0.16$), was significantly less with antibody GP71 than with M1 ($p < 0.02$, $p < 0.02$ and $p < 0.05$, respectively).

The concentration of the second motilin peak ($K_{av} 0.67$) however was identical with all antibodies.

	GP 71		M 1	
	Peak I	Peak II	Peak I	Peak II
Antrum (n=5)	5±2	11±4	16±3.5	14±10
Duodenum (n=5)	34±12	66±21	84±22	61±19
Jejunum (n=5)	23.5±12	57±17	63±20	57±18

Table 306

The two immunoreactive motilin peaks (pmol/g)
(mean ± SEM) in three regions of the human gut using two
antibodies.

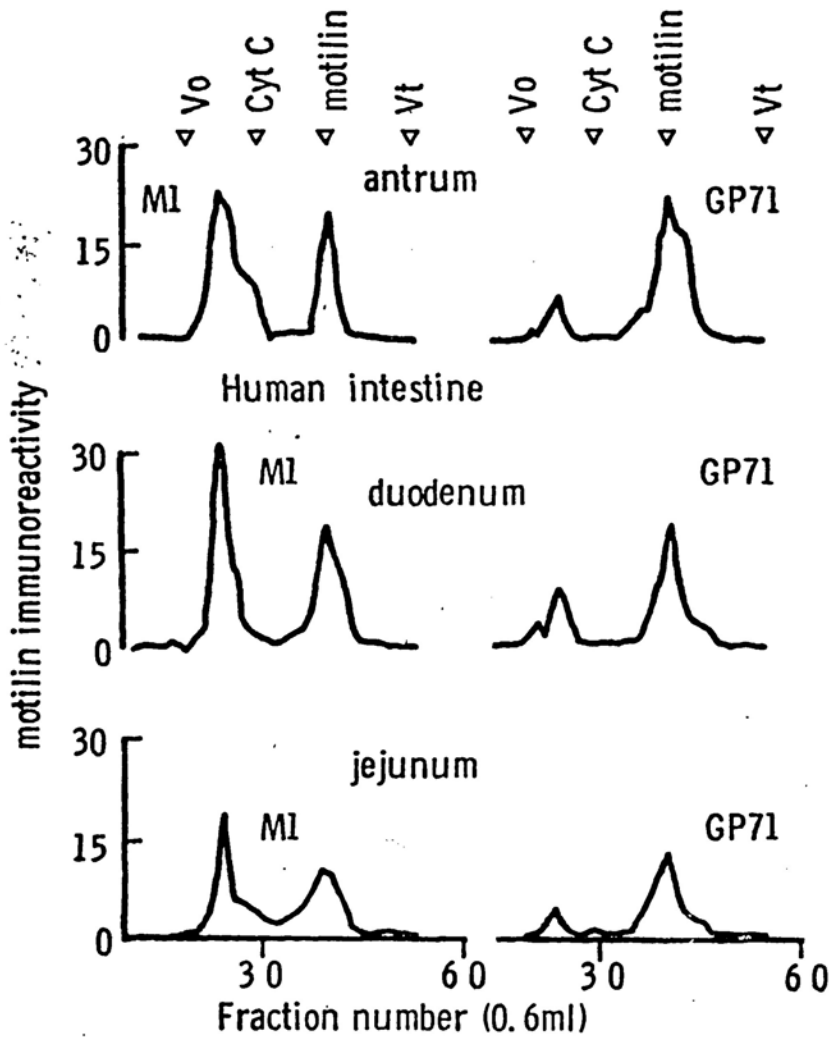


Figure 308

Elution profile of immunoreactive motilin from human antrum duodenum (middle), and jejunum (bottom) using antibody M1 (left) and antibody GP71 (right). The permeation analysis was on a Sephadex G-50 superfine gel column eluted at 6ml/h (0.6 ml fractions). The concentrations are expressed as fmol/assay tube.

Porcine tissues

In porcine intestinal extracts (Figure 309), in contrast to the findings in humans, the high molecular motilin ($K_{av}=0.16$) represented only a minor proportion ($2\pm 4\%$ $n=5$) of the total while the bulk of the motilin immunoreactivity was found in the position of the motilin standard ($K_{av}=0.67$). All three antibodies detected similar amounts of motilin immunoreactivity.

Canine tissues

The apparent motilin concentrations in canine intestine, as measured by antibody GP 71, are shown in the table 307. No apparent motilin was detected with M1.

Other species

Addition of up to 100 μ l of duodenal extracts from rabbit, rat, mouse and frog did not decrease the label binding of antibody M1 and GP 71.

Table 307

	<u>Motilin, pmol/g</u>
Antral mucosa	undetectable
Antral wall	103±5
Fundal mucosa	undetectable
Fundal wall	39±3
Duodenal bulb mucosa	61±3
Duodenal bulb wall	176±28
2nd and 3rd part of duodenal mucosa	66±4
2nd and 3rd part of duodenal wall	156±12

Table 307

Motilin immunoreactivity (mean ± SEM, pmol/g) in canine intestinal extracts.

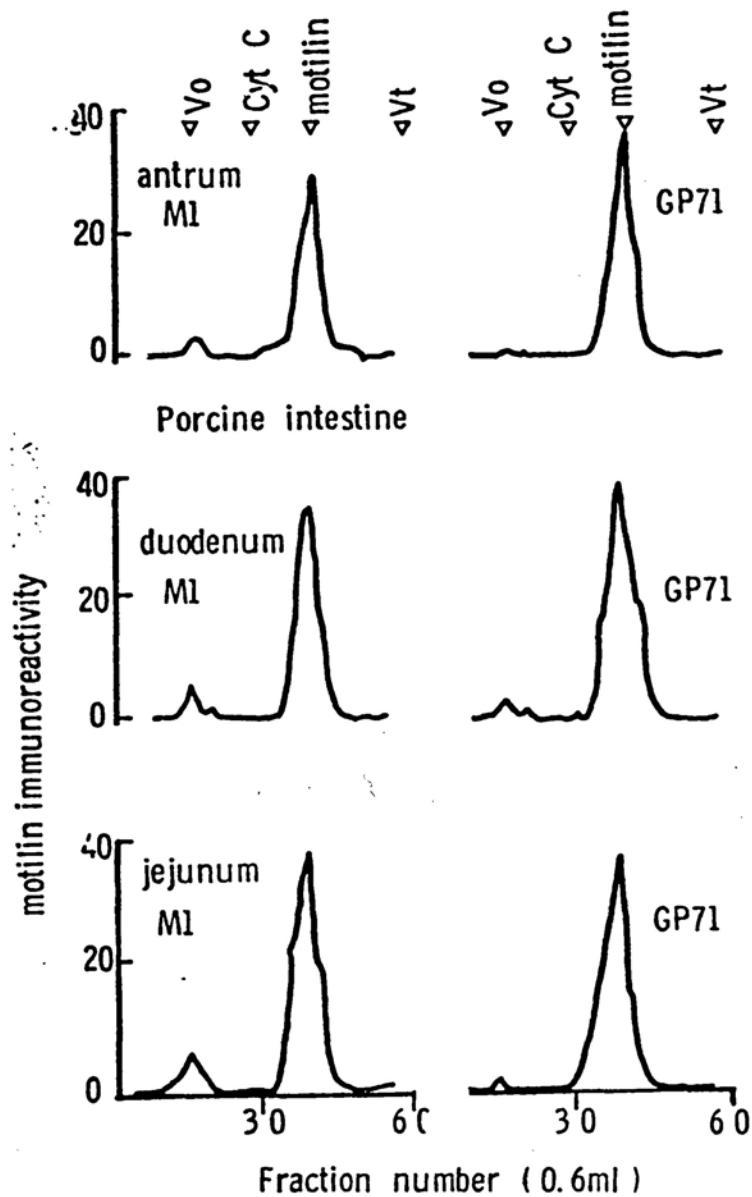


Figure 309

Elution profile of immunoreactive motilin from porcine antrum (top), duodenum (middle), and jejunum (bottom) using antibody M1 (left) and antibody GP71 (right). The permeation analysis was on Sephadex G50 superfine gel column eluted at 6ml/h (0.6ml fractions). The concentrations are expressed as fmol/assay tube.

DISCUSSION

The study of a physiological role of any candidate hormone (73) requires its measurement of the dynamics of its release. The only technique that is sensitive and specific enough to measure the minute amounts of the hormone in the circulation is radioimmunoassay. The development of a radioimmunoassay for motilin proved to be a relatively easy task. Antisera to porcine motilin, conjugated by either glutaraldehyde or carbodiimide to bovine serum albumin, were raised in both rabbits(22, 74)and guinea pigs(36,75,76,77) Porcine or synthetic motilin has been iodinated using either the chloramine T or lactoperoxidase oxidation methods and the labelled motilin demonstrated a high specific activity (63-76 Bq/fmol). Both the motilin label and the endogenous motilin appear to be relatively stable in plasma. Removal of all apparent motilin immunoreactivity by affinity immunoabsorption suggested that there is little non-specific protein interference to the antibody binding. This was further implied by Scatchard plot analysis, revealing no significant alteration of the affinity constant by plasma.

The antisera produced, showed total specificity towards motilin with no crossreaction with any of the other gastrointestinal hormones tested. These antibodies, however, exhibited different regional specificities. Antibodies GP 71 and M25 appeared to be C-terminal specific, whereas antibody M1 crossreacted strongly with the N-terminal region. The importance of using regional specific antibodies has been highlighted in the chromatographic analysis of tissue extracts. Thus, it was shown that the N-terminal specific antibody, M1, detected two major forms of motilin immunoreactivity, whereas antibody M25, detected only one; antibody GP71 detected two peaks, although peak I was only one third the value detected by M1. These results suggested the possibility of the existence of a large molecular form of motilin, that may have a C-terminal 'elongation' on 22 amino acid peptide (see also chapter 4).

Although the sequence of motilin from species other than porcine is not known, it is apparent that there is a large

species difference. Dog motilin is only measureable with C-terminal specific antibodies, suggesting differences in the N-terminal sequence. The motilin from the other species tested appear, if present, to be even more different from the porcine sequence, as none of the antibodies crossreacted with it.

As previously mentioned the radioimmunoassay does not measure biological activity; it merely quantitates a specific aminoacid sequence. It is thus important to know whether a particular antibody reacts with the biologically active region of the hormone. In vitro studies on isolated smooth muscle preparations using synthetic motilin fragments, revealed that the biologically active region of motilin resides in the N-terminal part of the molecule (20,21). Thus, antibody M1, not only detects an additional molecular form of motilin but also reacts with the biologically active region. For this reason this antibody has been used for measurement of motilin in plasma (Chapter 4).

Several radioimmunoassays for motilin have been reported in the literature (22,74,75,76,77). The methodologies employed in each case are broadly similar. These investigators have used antibodies raised to natural porcine motilin or synthetic motilin conjugated to bovine serum albumin or other carriers, such as polyvinyl pyrrolidone, by carbodiimide conjugation, and prepared the iodinated motilin tracer by chloramine T oxidation. The majority of investigators employed a Sephadex gel chromatography label purification step with the exception of Chey et al who used ion exchange chromatography (on C M-Sephadex C-25 eluted with 300 mM/l ammonium acetate, pH5.9)(74). The specific activities of the motilin tracer reported are very similar. The difference in the methodologies between the various laboratories only occurs in the plasma assays. Track et al (76) do not use motilin-free 'zero' plasma in the standard curve while Chey et al (74) employ an extraction procedure (because of the susceptibility of the antibody to non-specific protein interference). It is clear, on theoretical grounds, that the values obtained when 'reading' a plasma sample from a buffer standard curve the results are likely to be erroneous. An example, using

an antibody (M26) that is interfered with by non specific plasma protein effects is shown in fig 310. The 'zero' binding (ie the percentage of label bound by the antibody in the absence of standard) in the buffer system was 50%. Addition of 200 μ l of the motilin free plasma resulted in a drop of the binding to 27%. The percent bound of the unknown sample was 22.5% which, when 'read off' the buffer standard curve suggested a concentration of 51 pmol/l whereas it represented only 15 pmol/l when 'read off' the plasma standard curve. Extracting motilin from plasma, diminishes these non-specific effects. This method however, is considerably more tedious and could introduce errors, such as denaturation of the hormone or loss of immunological activity, variable extraction recoveries and concentration of non-specific inhibitors or proteolytic enzymes.

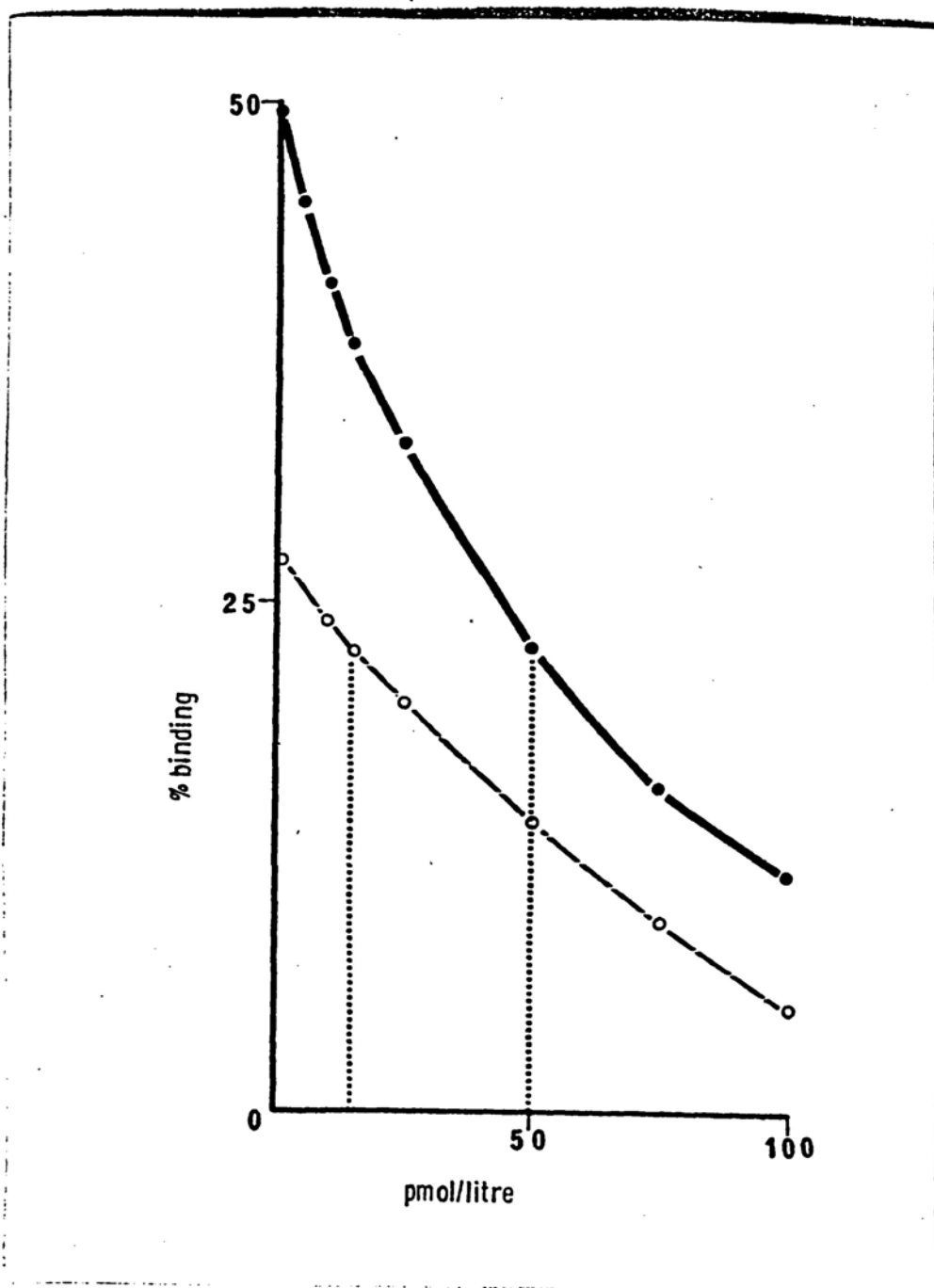


Figure 310

Dose-response curves, in buffer (solid dots) and in the presence of 200 μ l of zero plasma (open dots), using antibody M26. The percent binding of the unknown sample was 22.5% which corresponded to 51 pmol/l when 'read off' the buffer standard curve, and 15 pmol/l, when 'read off' the plasma standard curve.

There has been only one detailed report of the study of the molecular heterogeneity of motilin in gastrointestinal tissue extracts (78). These investigators employed the use of an apparently C-terminal specific antibody and sephadex G50 chromatography (using 3M acetic acid as eluent) and revealed the presence of four motilin immunoreactive peaks in the human corpus, duodenum and jejunum. Peak IV eluting just after the porcine motilin standard represented a very small proportion of the total motilin immunoreactivity (<1%). Peak III, representing the bulk of immunoreactivity, co-eluted with the porcine motilin standard. Peak II (about 5% of the total motilin immunoreactivity) eluted after the void volume. Peak I was only present in the duodenum and jejunum and eluted in the void volume. These results are broadly similar to those presented here. There are however some minor differences. Antibodies M1 (N-terminal specific) and GP 71 (the C-terminal specific) measured two immunoreactive peaks corresponding to the peak II and peak III of Yanaihara et al (78). Peak IV appeared to be below their reported assay detection limit (at the 5% level) and thus it may represent assay 'noise'. It could, on the other hand, be real and represent a minor small molecular size motilin that is measurable only with some antibodies; although both, antibody GP 71 and Yanaihara's R1105 are, broadly speaking, C-terminal specific there are however some differences. Antibody R1105 cross-reacted by 69% with the motilin fragment 7-22 and by 115% with fragment 12-22, whereas the corresponding values for antibody GP 71 were 55% and 7% respectively. It is thus possible that the reason for the discrepancies is the use of antibodies of different regional specificities. The presence of an immunoreactive motilin peak eluting in the void volume, picked up by antibody R1105 but not with GP 71 or M1 or M25, is difficult to explain. Again the reason may lie on the antibody specificity; on the other hand this immunoreactive peak may represent a non-specific protein interference of the antibody. A similar 'void volume' peak was reported, using a pancreatic glucagon antibody, by Weir et al (79), but it was subsequently shown to be due to non-specific interference. These results suggest the presence of several

immunoreactive motilins, that may represent a motilin family of peptides. It is clear that these peptides may differ from the classical porcine motilin sequence and thus they may be measured by some antibodies but not by others. Isolation and purification of these peptides is now clearly warranted.

There have not been many detailed studies on the distribution of motilin. Bryant et al (80) reported that in the baboon motilin was distributed in the upper intestine and the concentration in the duodenum was 100-150 pmol/g (similar to the human and canine distributions reported here). Dryburgh et al (75) also reported similar values in the canine intestine. Yanaihara et al (77) and Chey et al (81) however reported values considerably lower (3pmol/g and 0.4pmol/g respectively). The reason for this large discrepancy is difficult to reconcile although it could be because of the use of antibodies that poorly crossreacted with canine motilin.

CHAPTER 4

RELEASE OF MOTILIN

Introduction

The existence of the substance which later became known as motilin was postulated because of studies which demonstrated that alkalinisation of the duodenum of the dog induced an increase in motor activity in pouches of the body of the stomach (9). Further studies with this animal model demonstrated that the induced increase in gastric pouch motor activity by duodenal alkalinisation could be prevented if the duodenum was pretreated by perfusion with emulsified olive oil, peptone solutions, acetyl choline or hydrochloric acid.

The development of a radioimmunoassay for the measurement of motilin in plasma enabled Dryburgh et al (75) to test the hypothesis of a motilin release following alkalinisation of the dog duodenum. Indeed it was found that duodenal alkalinisation produced a transitory elevation of plasma immunoreactive motilin. This elevation of immunoreactive motilin coincided with an increase in gastric motor activity. Introduction of saline into the duodenum was without effect on both plasma motilin and gastric pouch motor activity. Introduction of acid into the duodenum resulted in a significant increase in plasma motilin without an accompanying change in gastric motor activity (75,82). The levels after alkali appeared to be higher and more rapidly elevated than when acid was perfused. These authors (82) postulated that there may be a threshold motilin level below which the peptide is ineffective as a motor activity stimulant or that acid in the duodenum releases both motilin and inhibitory substances for motor activity which negate the stimulatory action of motilin.

Subsequent studies in man reported that motilin does rise following duodenal acidification (83,84) although this elevation appeared to depend on the acid instillation rate (84). However, in contrast to the findings in dogs, duodenal alkalinisation in man (83), resulted in a reduction of plasma immunoreactive motilin. Release of motilin following duodenal acidification has also been reported to occur in the pig (85). The effect of alkali on this species, however, has not been studied.

An important step in the investigation of the physiology of any new hormone is the study of its release in the circulation. This chapter describes the studies that were undertaken to elucidate the mechanisms of motilin release.

Patients and methods

Blood collection

A 19G butterfly needle was inserted in an antecubital vein and blood was withdrawn in a plastic syringe. The butterfly line was kept patent by reinfusing 0.15mol/l saline. The blood samples were transferred into 10ml lithium heparin containers, gently mixed and then spun at 800g for 10-15 min. The plasmas were then separated and rapidly frozen.

Intraduodenal Stimulation

Acid

Nine healthy human volunteers, aged 23-36 years were studied after an overnight fast. Each subject gave fully informed consent for the test (carried out at the University of Erlangen, Germany). A 16F Levine tube was placed with its tip in the second part of the duodenum under X-ray control and, after a 30 min basal period, 50ml of 0.15mol/l saline was instilled into the duodenum over 3 min. This was followed 30 min later by 50 ml 0.1mol/l hydrochloric acid, also infused over 3 min. Blood samples were collected at 5 min intervals during the basal period, at 1 min intervals over first 10 min during and after each infusion, and thereafter every 5 min for a further 20 min.

Alkali

Intraduodenal alkalinisation was performed in 6 subjects. The same methodology was used as for the acid instillation. After a basal period of 30 min either 50ml of 0.15mol/l saline or 50ml of 0.3Mtris buffer (pH 10.2) were instilled intraduodenally over three minutes. Blood samples were taken at 5 min intervals during the first 10 min after instillation and thereafter at 5 min intervals for 30 min.

Oral stimulation

Meal

A standard meal containing two boiled eggs, orange juice, buttered toast and marmalade, composed of 66g carbohydrate, 18g protein and 22g fat (530 calories, 200ml) was given after an overnight fast to 31 subjects, 23 men and 8 women ($103 \pm 3\%$, mean $\pm$ SEM, of ideal weight aged 23-62 years). Blood samples were taken at 30 min, 15 min and just prior to ingestion

and at 15,30,60,120 and 180 min following ingestion. The motilin concentration in these samples was measured on five separate occasions and the interassay variation was calculated. The mean level of these was used when comparing the 'normal' response to a meal with the responses in various diseases (see chapter 6).

Another group of subjects ingested a test breakfast during the refractory period following a duodenal activity front. Their motilin response was compared to a further group of subjects that remained fasted and acted as controls. The first group consisted of 8 subjects (7 men and 1 woman aged 14-61 years). After an overnight fast motility recordings were started and continued until an activity front was observed. Immediately after an activity front had passed the distal recording orifice (time 0) a continental breakfast of 450 Calories was administered. Blood samples were collected at time 0 and at 15,30 and 45 min following the ingestion of the breakfast. The control group consisted of 12 subjects (11 men and 3 women, aged 14-65 years). The same protocol was followed, with the exception that the test breakfast was not given to this group.

The upper intestinal motility was measured by means of 3 perfused polyethylene catheters (PE 205, Clay Adams, Div of Becton, Dickinson and Co., Parsippany, N.J.) with recording orifices 25cm apart (all motility measurements were carried out at the Department of Medical Research, Leuven, Belgium)(86). The proximal orifice was positioned under fluoroscopic control in the distal part of the duodenum about 10 cms above angle of Treitz. Details of the methods of recording and of analysis of the pressure records are described in chapter 5.

Glucose.

Fifty seven subjects, 40 men and 17 women ($103 \pm 5\%$ of ideal weight) aged 20-61 years ingested 50gms of glucose (198 Calories, 250ml). Blood samples were taken at 0,30,60,90 and 120 minutes following the glucose.

Fat

Seven subjects, five men and two women ($104 \pm 5\%$ of ideal weight) aged 21-35 years (mean 27 years) were given 120ml double cream made up to 500ml with water (515 Calories). Blood samples were taken 20 min prior to, and at 0,30,60,90 and 120 min following the ingestion of the double cream.

Protein

Six subjects, four men and two women ($105 \pm 5\%$ of ideal weight) aged 22-35 years (mean 27 years) were given 680g steamed cod (515 Calories, 500ml). Blood samples were taken 20 min prior to, and at 0,30,60,90 and 120 min following the ingestion of the protein.

Water

Fifteen ml of distilled water (temp 20°C) per Kg body weight was given orally to 6 subjects, all male ($106 \pm 8\%$ of ideal weight), aged 22-35 years (mean 27 years). The experiment was repeated two weeks later on 5 of these subjects, who were given 600 μg atropine intravenously 2 min prior to the ingestion of the water load (15ml/kg). A different group of 6 subjects, all male ($103 \pm 5\%$ of ideal weight), aged 20-30 years (mean 24 years) were given 7.5ml water per Kg body weight. The ingestion time was always less than 5 min. Blood samples were taken 20 min and 10 min prior to and at 0,5,10,20,30,40,50 and 60 min following the ingestion of water.

Antral balloon distension

Nine subjects, aged 22-25 years, participated in these studies performed by Dr I.M. Schoon, University of Goteborg, Sweden. The antral balloon distension was performed as follows: A small thin-walled latex balloon was firmly tied to a polyethylene tube 3mm diameter that was attached to a double lumen nasogastric tube (Salem sump No 14). The position of the nasogastric tube was controlled by fluoroscopy of the X-ray visible thread in the Salem tube (87). After 30 min 'basal' an intravenous injection of either saline, metoprolol (0.2mg/Kg) or propranolol (0.1mg/Kg) was given. The injections were given in coded, randomised order. Thirty minutes after intravenous injection, the balloon was filled with 150 cc water solution of roentgen contrast medium (Gastrografin). The 'intraballoon' pressure was calculated to be about 32cm water. Blood samples were taken 30 and 15 min prior to and at 0,15,30,45,60 and 90 min after the start of the distension.

Intravenous stimulation

Nutrients

Four subjects, all men ($103 \pm 2\%$ of ideal weight) aged 22-30 years (mean 25 years) received the following 30 min isocaloric intravenous infusions in random order on separate days :-

- a. glucose (20g as 50% solution)
- b. amino acids (25g as 250ml of 10% Aminosol, Kabivitrum, Ltd)
- c. fat (8g as 40ml of 20% Intralipid, Kabivitrum, Ltd)

Arginine (0.5g/kg/hour) was given as a 30 min intravenous infusion to 22 subjects, 14 men and 8 women ($104 \pm 3\%$ of ideal weight) aged 25-52 years (mean 35 years).

Insulin

Two different protocols were employed :-

- a. A group of 6 subjects ($102 \pm 3\%$ of ideal weight) aged 23-35 years (mean 28 years) were given insulin (0.2 units/Kg) intravenously. Prior to the test a Ryles tube was inserted and gastric juice was drained throughout and discarded.
- b. A different group of 8 subjects ($104 \pm 6\%$ of ideal weight) aged 25-34 years (mean 28 years) were given 0.2 units/Kg of insulin. In this group of subjects the gastric juice was not drained.

Pancreatic polypeptide

After an overnight fast an antecubital vein was catheterised and 0.15M saline with human albumin (200 μ mol/l) was infused from a syringe ram pump (1 ml/min) for a basal period of 20 min. Highly purified bovine pancreatic polypeptide (BPP, Lilly Research Laboratories, batch No 615-D63-188-8) was then added to the infusate at doses of 1.19 ± 0.15 pmol/Kg/min (n=7) and 4.2 ± 0.38 pmol/Kg/min (n=10) for a period of 1h and then infusion of saline alone was resumed for a further period of 1 h. The subjects were healthy, aged between 23 and 45 and within 10% of ideal body weight.

Bombesin

Six subjects, all men, aged between 25-37 years, within 10% of ideal weight participated in this study. Synthetic bombesin (amphibian sequence) was infused, through an antecubital vein, at a dose of 0.3pmol/Kg/min on one day and 1.2pmol/Kg/min on another day. After a basal period of 30 min, bombesin (in 0.15M saline containing 200 μ mol/l human albumin) was infused for 45 min and then saline alone was infused for a further 45 min.

Somatostatin

Seven healthy fasting volunteers, six male and one female (aged 20-35 years), of normal weight participated in this experiment. Two indwelling cannulae were inserted into veins, one in each arm. Three blood samples were taken at 10 min intervals from one arm and the continuous infusion was then started into the contralateral arm. Blood samples were further collected at 10 min intervals for the following 80 min. Somatostatin (obtained from Clin Midy, Rue du Prof Joseph Blayac, 34082 Montpellier Cedex, France) was infused at a dose of 50 pmol/Kg/min.

Atropine

Six fasting subjects aged 25-35 years, of normal weight, were given 600 μ g of atropine intravenously. Blood samples were taken at -30,0,15,30,45,60,90,120,150,180,210 and 240 min after atropine.

Motilin during pregnancy and early life

Thirty seven women participated in this study. Nineteen of these were within 10 per cent of ideal body weight whereas the other eighteen were at least 20 per cent in excess of ideal body weight. These subjects were all recruited at the antenatal clinics held at St Mary's Hospital, London, under the care of Professor R.W. Beard. Each subject was studied at 16 and 36 weeks of pregnancy and one week postpartum. Following an overnight fast the subjects were given a conventional 3-hour 50g glucose tolerance test with half-hourly blood sampling. After this the subjects were given a mixed meal (66g carbohydrate, 18g protein and 22g fat), and further half-hourly samples were

taken over a period of 3 hours.

Sixty three preterm infants (birthweights 1950 ± 30 g mean $\pm$ SEM) and gestation 33.5 ± 0.1 weeks) and 45 term infants (birthweights 3312 ± 50 g and gestations 39.5 ± 0.1 weeks) were studied, with the approval of the ethics committee. Each infant contributed only one venous blood sample; this was taken either at birth (cord blood, at normal vaginal delivery) or postnatally. Postnatal samples were obtained from the back of the hand using an open needle technique, and were taken only at a time when blood was also required for clinical monitoring. Term infants were sampled either before the first feed at age 4-6 hours, or on day 6 (range 5-7), or day 16 (range 12-24); preterm infants were sampled before the first feed at age 3-6 hours, or on day $2\frac{1}{2}$ (1-4), day 6 (5-7), day 13 (11-17), or day 24 (18-42). In each group samples were taken immediately before feeding. Term infants were breast-fed over 25-35 minutes at 4-hourly intervals. Preterm infants were fed pooled human milk over 5 min via a nasogastric tube. Infants age $2\frac{1}{2}$ days were fed 2-hourly; infants aged 6, 13 and 24 days were fed 3-hourly.

In addition, 10 preterm infants were studied (ages 6.2 ± 0.5 days, birthweights 1875 ± 243 g, gestation 33 ± 1 weeks) who had hyaline membrane disease and lacked the ability to tolerate enteral fluids: they had received only intravenous dextrose since birth. They required ventilatory assistance and enriched inspired oxygen but each had a PaO_2 between 60 and 90 mmHg (7.9-11.9 kPa), $pH > 7.25$, and each was clinically stable at the time of the study.

Molecular forms in plasma

Chromatography

One ml plasma aliquots were applied to the top of 0.9x60 cm Pharmacia column containing 30ml Sephadex gel, G50 superfine. Columns were run at 6ml per hour at 40°C and were precalibrated with dextran blue, cytochrome C, sodium I^{125} and porcine motilin. Dextran blue and cytochrome C were included with each sample and ran as internal markers. Phosphate (0.06M buffer containing 0.01 M EDTA and 2% human serum albumin pH 7.4) was used as the elution buffer. Six minute fractions were collected and assayed for their motilin content. The undiluted plasmas were included in each radioimmunoassay run, for calculation of recovery :-

(Recovery% = $\frac{\text{Motilin immunoreactivity eluted from the column}}{\text{Motilin immunoreactivity applied to the column}} \times 100$).

Subjects

A total of 34 subjects (aged 21-55 years) of normal weight and no history of any gastrointestinal disease were studied. Two plasma samples (one basal and one after stimulation) from each subject were analysed after various stimuli :-

1. Standard breakfast containing 66g carbohydrate, 18g protein and 22g fat (n=8, 0 and 15 min)
2. Water load (7.5ml of distilled water per kg body weight, n=6, 0 and 20 min)
3. Oral fat (200ml of double cream containing 44% fat, made up to 500ml with water, n=5, 0 and 30 min).
4. Oral glucose (200ml of 50% glucose, n=5, 0 and 90 min)
5. A 90 min intravenous infusion of 50pmol/Kg/min of somatostatin (n=5, 0 and 75 min)
6. A 90 min intravenous infusion of porcine motilin (0.2pmol/Kg/min, n=5, 0 and 75 min).

All chromatographic fractions were analysed with antibody M1.

As mentioned in the previous chapter antibody M25 did not crossreact with peak I motilin ($K_{av}=0.16$) of the human intestine. Thus, if this also occurs in plasma, it would be theoretically possible to estimate this motilin peak by a simple subtraction of the value obtained with M25 from the motilin level seen with antibody M1. In order to validate this hypothesis 26 plasma

samples were chromatographed and the fractions eluted were examined with antibody M1, (and the value of both motilin peaks was thus estimated). The plasma motilin level (in neat plasma) was then measured using both antibodies and the correlation coefficient was thus examined. Also, in order to confirm the lack of crossreaction of M25 with peak I, seven plasmas were chromatographed and the fractions eluted were measured with M25.

RESULTS

Intraduodenal stimulation

Acid

Figure 401 and table 401 show the plasma motilin levels following intraduodenal instillation of saline and hydrochloric acid. The pre-saline mean motilin value was 23 ± 6 pmol/l. This level was not significantly altered by the saline (levels oscillating from 21 ± 6 to 26 ± 7 pmol/l). The motilin level rose sharply following duodenal acidification, the peak being achieved 5 min after instillation (46 ± 10 pmol/l, $p < 0.001$).

Alkali

As can be seen from Figure 402 no significant change in plasma motilin was seen after administration of the Tris buffer, although levels tended to decrease.

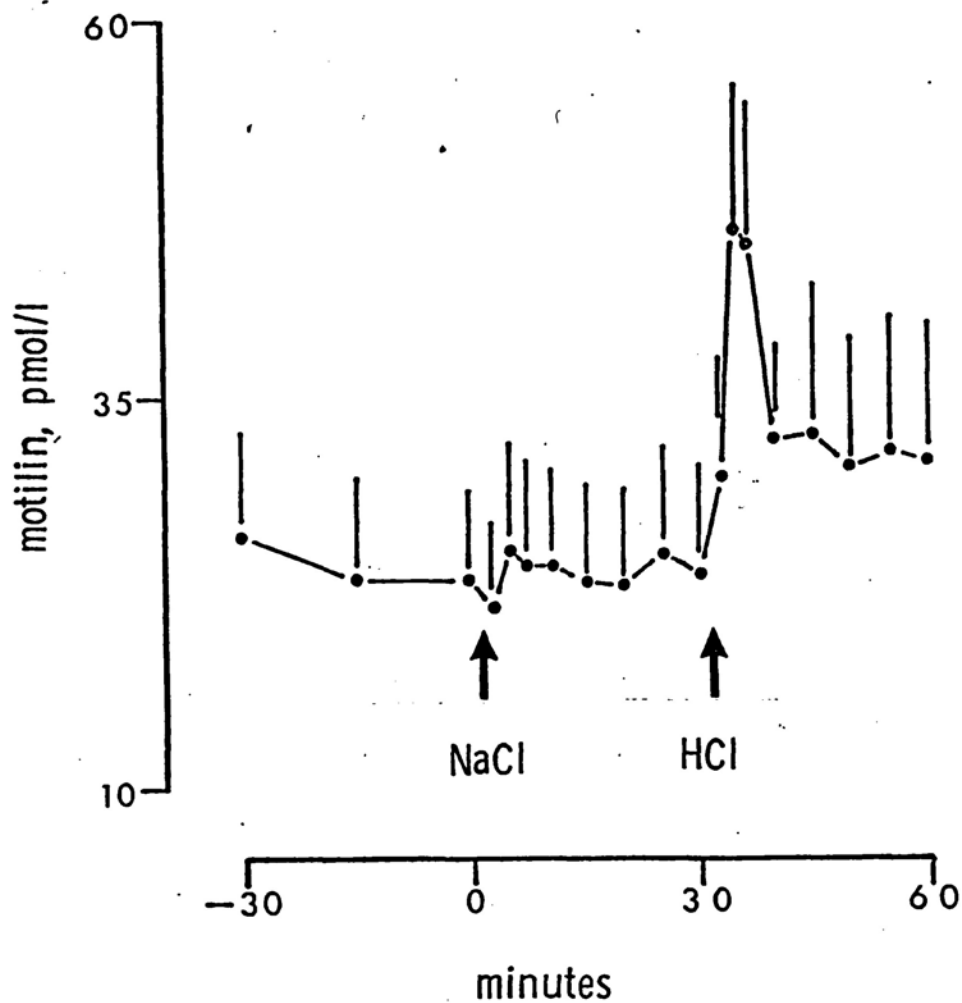


Figure 401

Plasma motilin (pmol/l) following saline (at time 0) and 50 ml of 0.1 mol/l hydrochloric acid (at time 30, given over 3 min).

Saline/Acid

	A	B	C	D	E	F	G	H	I	NAME	$\bar{X}$	SEM
-30	17	64	26	26	13	54	8	10	16	26		7
-15	15	50	25	24	12	56	6	7	14	23.3		6.4
0	17	59	24	22	14	52	10	8	12	23.2		5.9
<u>Saline</u>												
3	11	54	26	23	16	40	6	6	10	21.3		5.8
4	11	59	26	24	20	58	10	10	14	25.8		6.9
5	12	68	23	24	21	49	12	8	8	25		7.2
6	10	52	17	25	18	55	6	7	10	22.2		6.6
7	10	54	20	31	16	57	8	6	13	23.9		6.9
10	10	50	22	35	22	54	7	5	14	24.3		6.4
15	10	60	24	26	17	43	5	8	13	22.9		6.4
20	9	50	21	24	17	54	7	10	11	22.6		6.2
25	11	58	24	28	16	64	6	7	10	24.9		7.7
30	9	68	18	28	15	50	6	8	9	23.4		7.7
<u>Acid</u>												
33	16	68	23	27	24	66	16	17	13	30		7.6
34	23	92	42	39	29	82	30	21	20	42		9.4
35	24	98	31	58	30	86	24	36	26	45.9		10
36	20	100	23	43	28	83	26	33	48	45.4		10.3
37	22	98	22	39	20	84	20	20	32	39.7		10.6
40	14	68	24	37	22	58	24	15	26	32		6.7
45	15	84	23	42	13	76	18	7	15	32.6		10.1
50	20	84	17	47	18	47	18	4	17	30.2		8.8
55	18	86	12	48	18	52	26	10	13	31.4		9
60	12	80	12	51	15	56	22	13	18	31		8.8

Table 401

Plasma motilin levels (pmol/l) in 9 subjects receiving a 3-min intraduodenal perfusions of saline (starting at time 0) and 0.1 N hydrochloric acid (starting at 30 min).

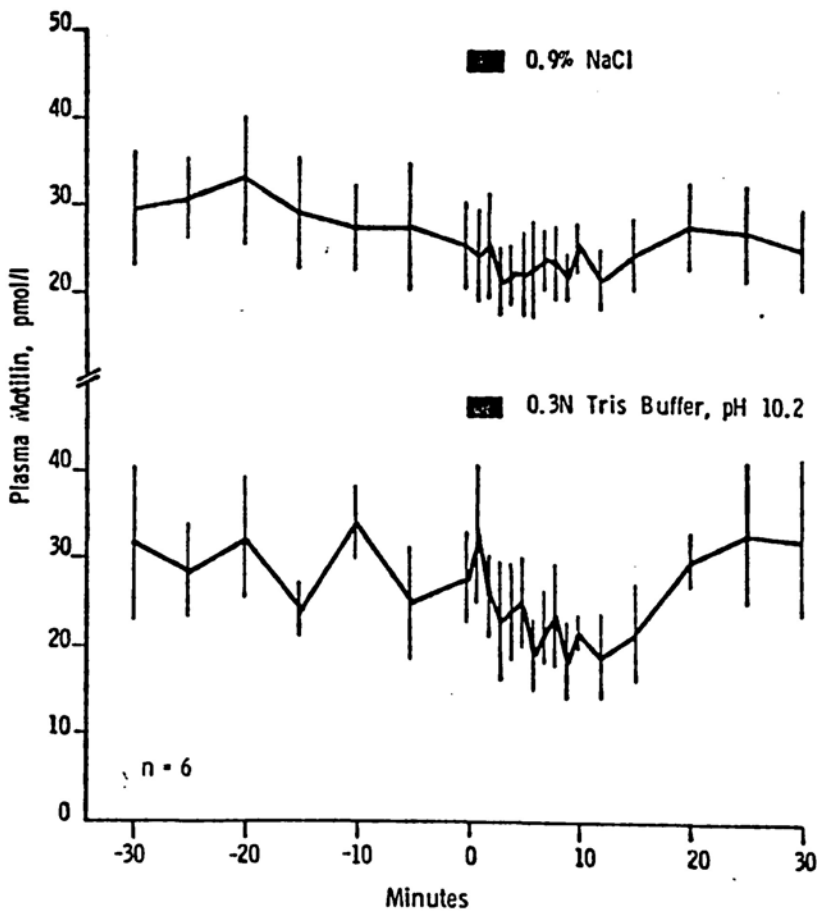


Figure 402

Plasma motilin (pmol/l) following duodenal alkalinisation.

Oral stimulation

Meal

Figure 403 and Table 402 show the mean effect of the meal (5 estimations) on plasma motilin levels. The interassay variation was calculated to be 7%. The pre-ingestion motilin value was 48 ± 6.6 pmol/l. Following ingestion, motilin levels rose significantly reaching a peak at 15 min (75 ± 10 pmol/l, $p < 0.001$). This rise was shortlived and levels quickly returned to pre-ingestion value within 1 hour.

Figure 404 shows the plasma motilin levels following the test breakfast, with simultaneous measurement of intestinal activity. Following ingestion of the breakfast there was a significant rise of motilin at 15 and 30 min ($p < 0.05$). These values were also significantly higher than the corresponding control values ($p < 0.01$ and $p < 0.02$ respectively).

Glucose

The mean pre-ingestion level was 46 ± 5 pmol/l (Figure 405 and Table 403). Levels fell sharply following ingestion of the glucose, the nadir being achieved at 90 min (19 ± 2 pmol/l, $p < 0.001$). Levels remained significantly suppressed at the end of the experiment (120 min, 20 ± 2 pmol/l, $p < 0.001$).

Fat

The effect of fat ingestion on plasma motilin is shown in Fig 406 and Table 404. Plasma motilin levels rose from a basal of 60 ± 12 pmol/l to 92 ± 9.5 pmol/l and 85.5 ± 10 pmol/l at 30 and 60 min after ingestion (both $p < 0.05$). They then started to fall returning to basal levels at 90 min.

Protein

The effect of protein ingestion on plasma motilin is shown in Fig 407 and Table 405. Plasma motilin levels tended to rise after the ingestion, although statistical significance was not reached at any point.

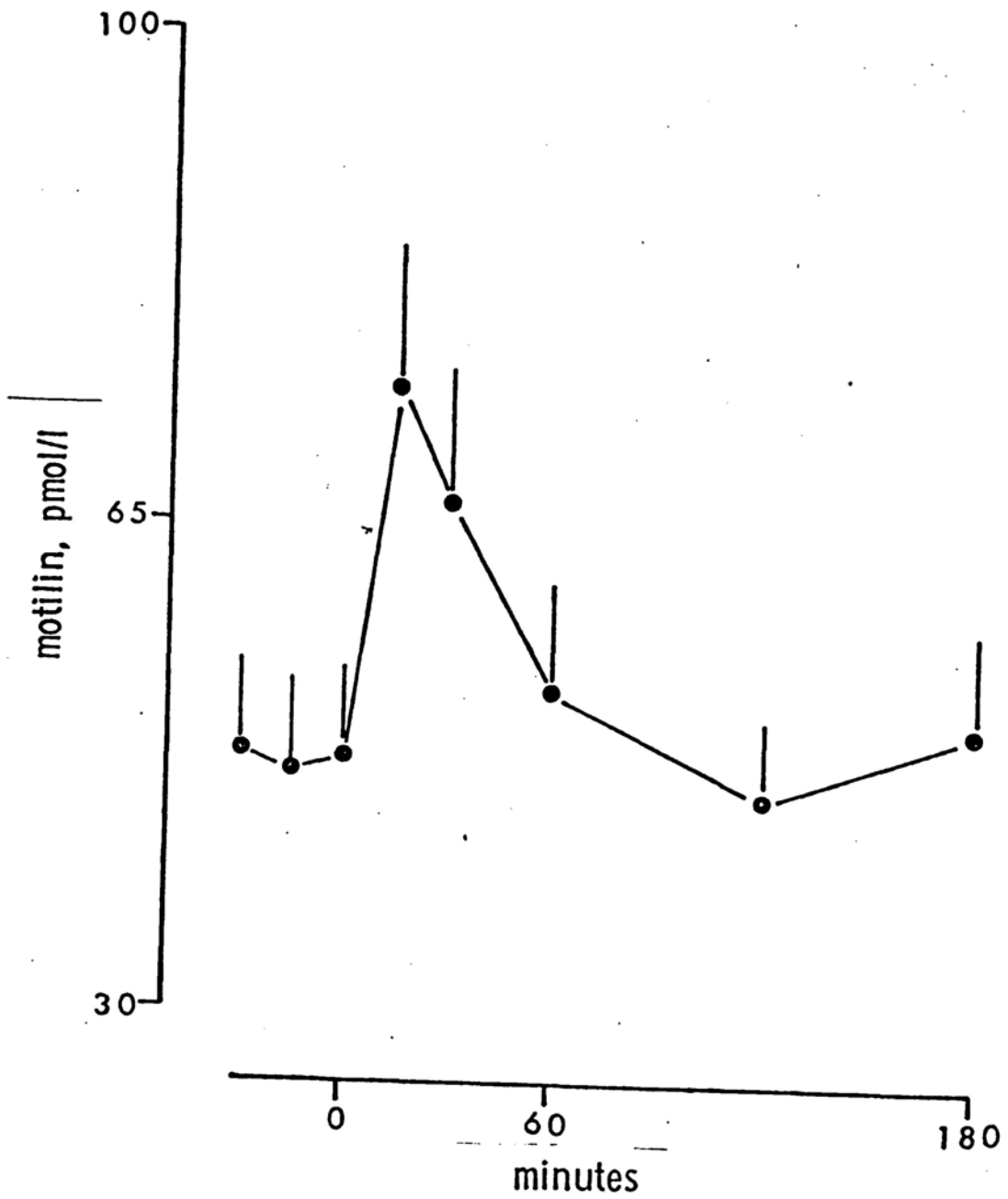


Figure 403

Plasma motilin (pmol/l) following ingestion of the test meal.

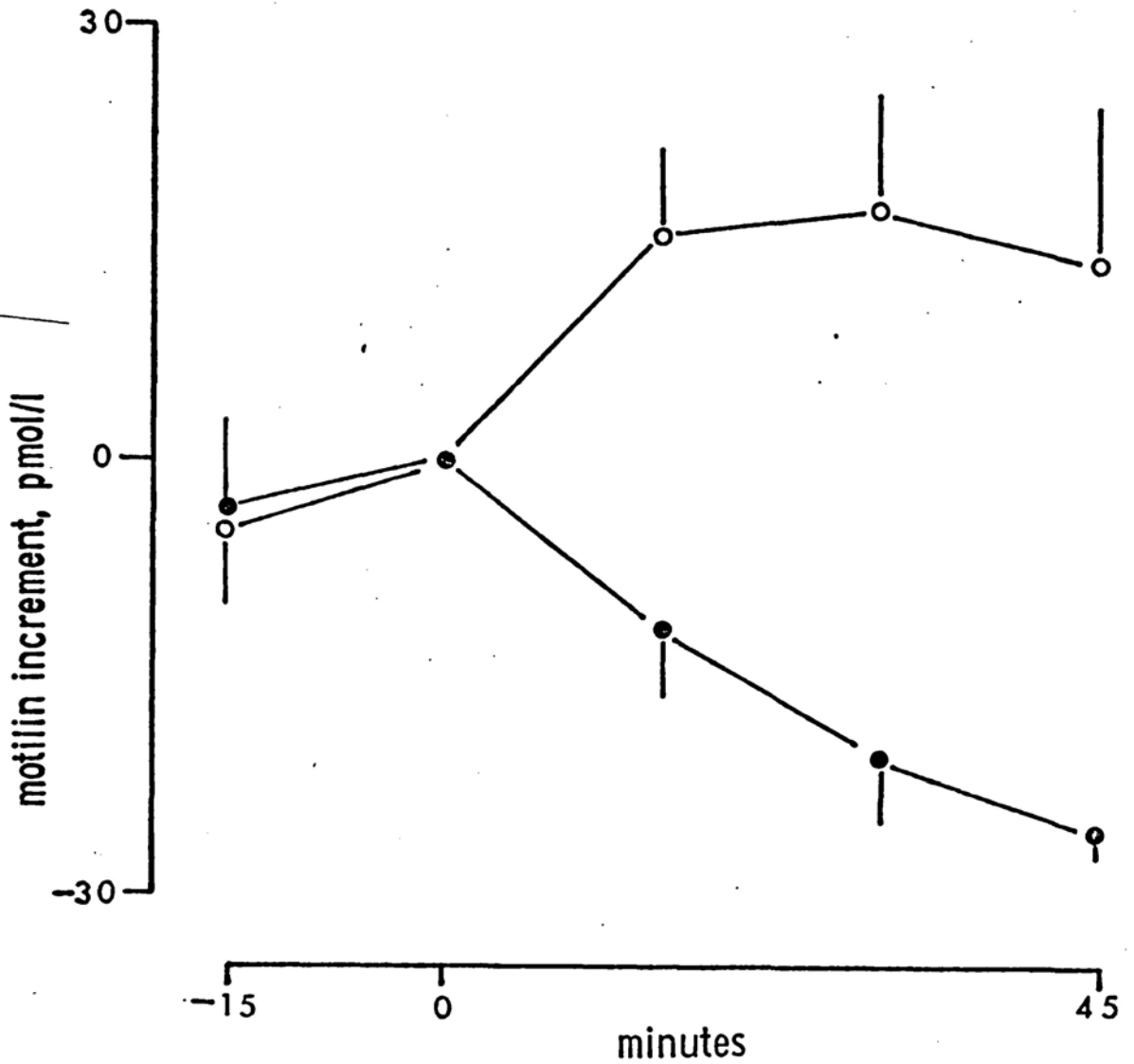


Figure 404

Plasma motilin change (pmol/l), following the breakfast in 8 subjects (at time 0, open circles). The controls (no breakfast ingested)(n=12) are depicted in solid dots. Time zero represents the time after an activity front had passed the distal recording orifice.

Subjects	-30	-15	0	15	30	60	120	180
1	14	13	12	39	41	26	20	22
2	18	19	18	49	40	31	24	37
3	12	13	12	62	31	25	25	31
4	41	55	44	84	74	62	59	60
5	93	88	99	167	163	130	108	136
6	50	43	59	88	83	66	77	70
7	6	9	5	7	3	3	2	3
8	111	128	128	136	124	75	46	72
9	89	81	80	108	132	130	110	82
10	91	85	93	121	130	110	103	99
11	10	10	14	26	22	22	23	24
12	56	45	50	84	50	36	34	52
13	104	110	106	91	87	57	56	37
14	26	17	23	37	33	27	20	20
15	41	33	44	58	60	31	34	38
16	71	68	72	96	85	71	64	69
17	30	39	32	40	54	49	38	27
18	36	36	37	52	32	25	18	20
19	18	13	16	41	28	34	38	39
20	32	30	37	66	77	67	55	65
21	12	14	12	21	23	19	13	13
22	17	18	15	28	33	27	24	25
23	12	13	11	22	18	15	14	16
24	78	74	74	79	64	65	63	89
25	61	53	55	97	99	74	48	48
26	32	27	35	42	28	29	24	34
27	118	115	93	189	130	77	76	85
28	18	14	12	12	11	11	13	15
29	112	112	129	259	230	187	120	169
30	39	40	32	48	33	29	28	38
31	59	49	45	67	46	25	22	30
X	48.6	47.2	48.1	74.7	66.6	52.9	45.1	50.5
SEM	6.4	6.5	6.6	10.1	9.3	7.6	5.8	6.8
log Transformation of raw data								
X	1.56	1.54	1.54	1.76	1.69	1.59	1.53	1.59
SEM	0.07	0.07	0.07	0.06	0.07	0.07	0.07	0.06

Table 402

Plasma motilin (pmol/l) in 31 subjects, following ingestion of the test meal.

Subjects	0	30	60	90	120
1	49	48	30	24	32
2	23	15	16	10	8
3	105	75	50	47	47
4	20	15	8	12	15
5	19	5	5	3	3
6	35	33	29	20	19
7	18	17	13	5	6
8	67	31	20	23	35
9	47	31	29	23	35
10	133	55	52	41	40
11	40	35	20	24	13
12	43	33	20	15	10
13	72	70	69	38	35
14	131	101	68	57	77
15	24	13	13	12	9
16	64	52	38	29	30
17	26	16	20	9	10
18	43	22	25	11	15
19	4	4	3	2	2
20	97	44	37	19	27
21	38	24	20	19	11
22	48	25	23	20	13
23	70	54	34	29	22
24	15	10	7	10	11
25	69	51	34	28	28
26	35	19	21	13	11
27	56	39	30	26	30
28	77	69	58	46	64
29	43	41	26	25	32
30	42	42	35	35	38
31	6	5	2	3	2
32	25	4	2	2	2
33	60	51	37	33	36
34	25	17	14	13	8
35	63	58	53	48	39
36	29	28	23	23	14
37	48	35	18	16	8
38	38	22	23	20	24
39	36	27	20	22	23
40	6	3	3	2	3
41	14	12	14	13	12
42	16	5	4	4	5
43	17	17	15	10	7
44	41	36	20	23	17
45	10	13	6	2	5
46	19	6	2	2	5
47	6	4	3	2	2
48	17	14	11	11	13
49	73	60	49	47	38
50	20	12	13	7	7
51	20	23	14	3	4
52	100	72	51	29	35
53	25	23	22	18	6

Subjects	0	30	60	90	120
54	180	67	43	20	44
55	152	62	53	33	37
56	14	2	3	4	2
57	20	8	7	4	4
X	46.2	31.1	24.2	19.1	19.8
SEM	5	3.06	2.34	1.86	2.2
<u>log transformation of raw data</u>					
X	1.55	1.34	1.23	1.12	1.12
SEM	0.05	0.05	0.06	0.06	0.06

Table 403

Plasma motilin (pmol/l) in 57 healthy subjects after ingestion of 50g of glucose.

Subjects	-15	0	30	60	90	120	180
1	59	65	90	111	82	115	76
2	98	81	101	95	99	92	88
3	64	57	85	75	47	47	44
4	31	19	90	84	69	78	82
5	19	23	70	56	40	54	40
6	50	52	70	58	49	35	36
7	104	118	138	119	98	84	72
X	60.7	59.2	92	85.4	69.1	72.1	62.6
SEM	12.9	13.9	9.5	10	10	11.5	8.9
<u>log transformation of raw data</u>							
X	1.72	1.71	1.95	1.92	1.82	1.82	1.77
SEM	0.11	0.12	0.01	0.05	0.07	0.07	0.07

Table 404

Plasma motilin (pmol/l) in 7 healthy subjects following ingestion of 120ml double cream.

Subjects	-15	0	30	60	90	120	180
1	48	44	62	59	64	68	59
2	47	38	45	59	53	56	57
3	61	61	59	63	56	56	60
4	94	108	152	148	150	134	144
5	90	90	97	89	97	86	99
6	48	43	56	57	54	55	70
X	64.6	64	78.5	79	79	75.8	81.5
SEM	9.8	12.9	17.9	16	17.2	13.8	15.4
<u>log transformation of raw data</u>							
X	1.79	1.77	1.86	1.87	1.86	1.85	1.88
SEM	0.06	0.08	0.09	0.07	0.08	0.07	0.07

Table 405

Plasma motilin (pmol/l) in 6 healthy subjects following ingestion of 680g steamed cod.

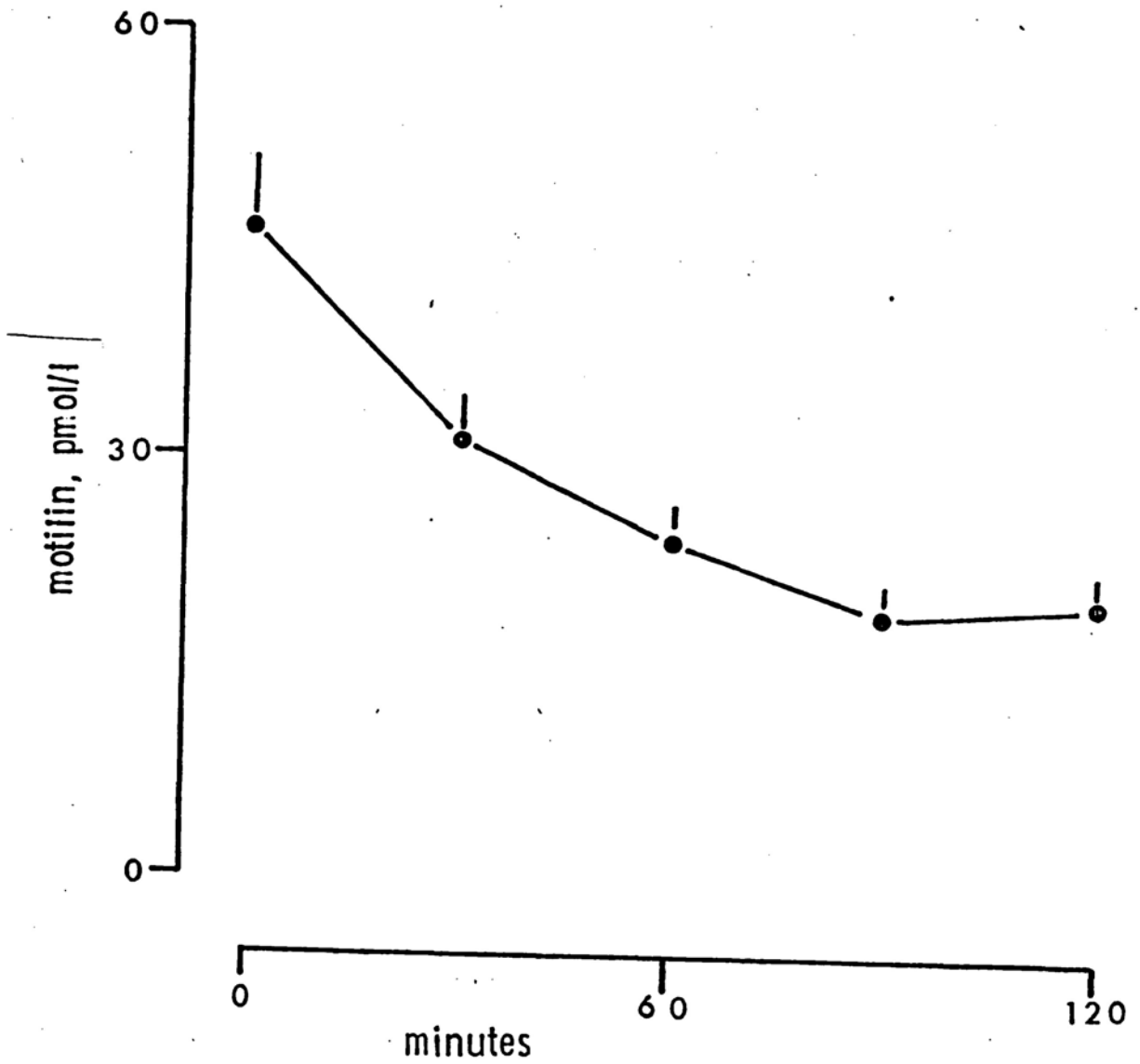


Figure 405

Plasma motilin (pmol/l) in 57 subjects, following ingestion of glucose

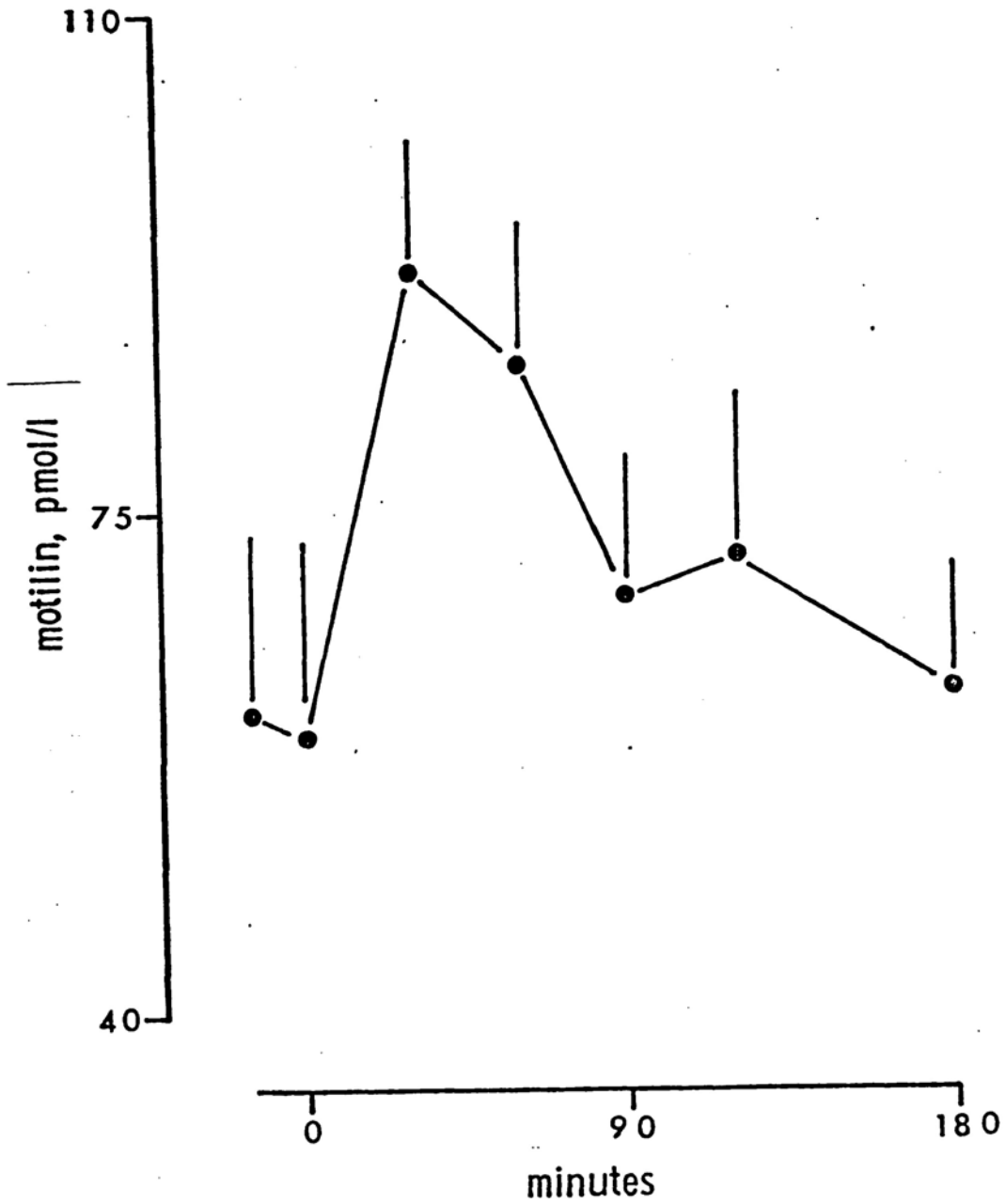


Figure 406

Plasma motilin (pmol/l) following ingestion of fat (double cream).

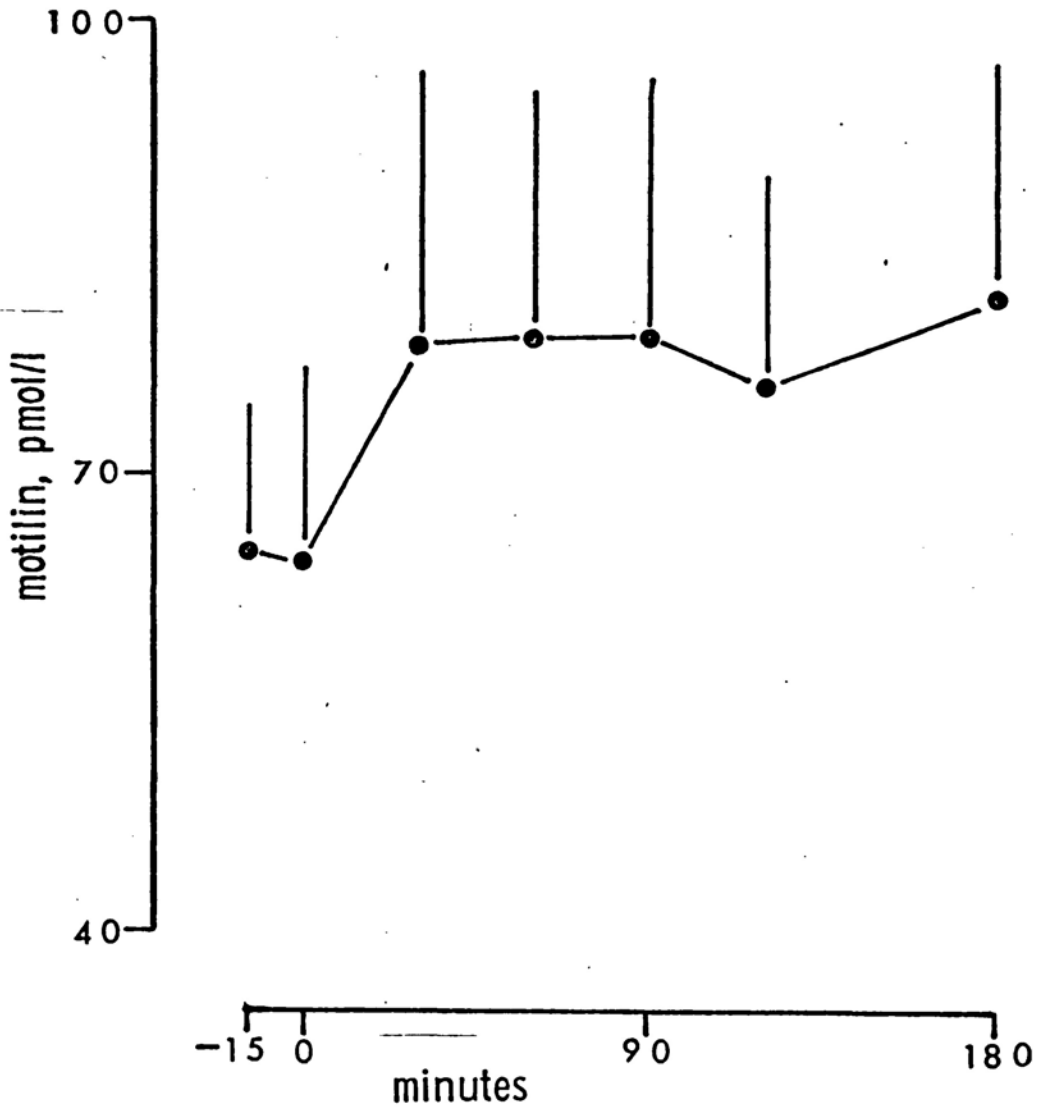


Figure 407

Plasma motilin (pmol/l) following ingestion of protein (steamed cod).

Water load

Figure 408 shows the response of plasma motilin following the ingestion of water and the effect of atropine. Following ingestion of the 7.5 ml/Kg of water load (bottom graph), motilin levels rose from a mean pre-ingestion value of 56 ± 12 pmol/l to a mean peak value at 20 min of 117 ± 30 pmol/l ($p < 0.005$). Levels remained significantly elevated for 40 min before returning to basal values at 50 min. Following ingestion of 15ml/Kg of water plasma motilin levels rose from a mean value of 50 ± 14 pmol/l to a peak at 30 min of 97 ± 25 pmol/l ($p < 0.01$). Levels remained significantly elevated up until the end of the experiment, the 60 min mean value being 95 ± 27 pmol/l. Following ingestion of the 15ml/Kg water load by the atropinised subjects, plasma motilin rose slowly, from a mean zero value of 52 ± 19 pmol/l to a peak level at 50 min of 105 ± 42 pmol/l ($p < 0.01$).

Antral balloon distension

Figure 409 shows the effect of antral distension, following injections of saline, metoprolol or propranolol, on plasma motilin. Plasma motilin rose following distension (saline group, bottom graph) from a mean value of 31 ± 9 pmol/l to a peak of 63 ± 10 pmol/l ($p < 0.01$). The corresponding rises in the metoprolol (35 ± 10 to 56 ± 10 pmol/l, $p < 0.02$) and propranolol groups (31 ± 7 pmol/l to 51 ± 11 pmol/l $p < 0.05$), although lower, were not statistically different from the control. The intravenous injections of metoprolol and propranolol had no effect on the basal motilin values.

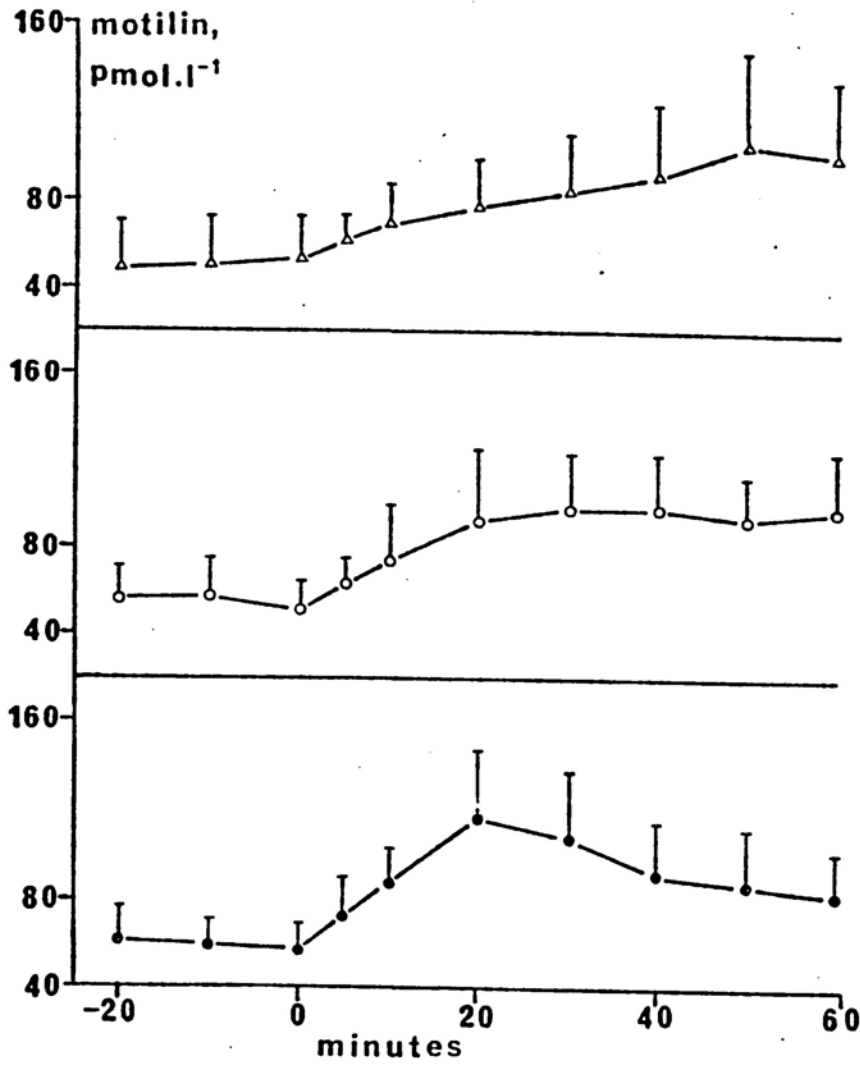


Figure 408

Plasma motilin concentrations (pmol/l) following ingestion of 7.5ml/Kg of water (bottom), 15ml/Kg (middle) and 15ml/Kg and intravenous atropine (top) by healthy subjects.

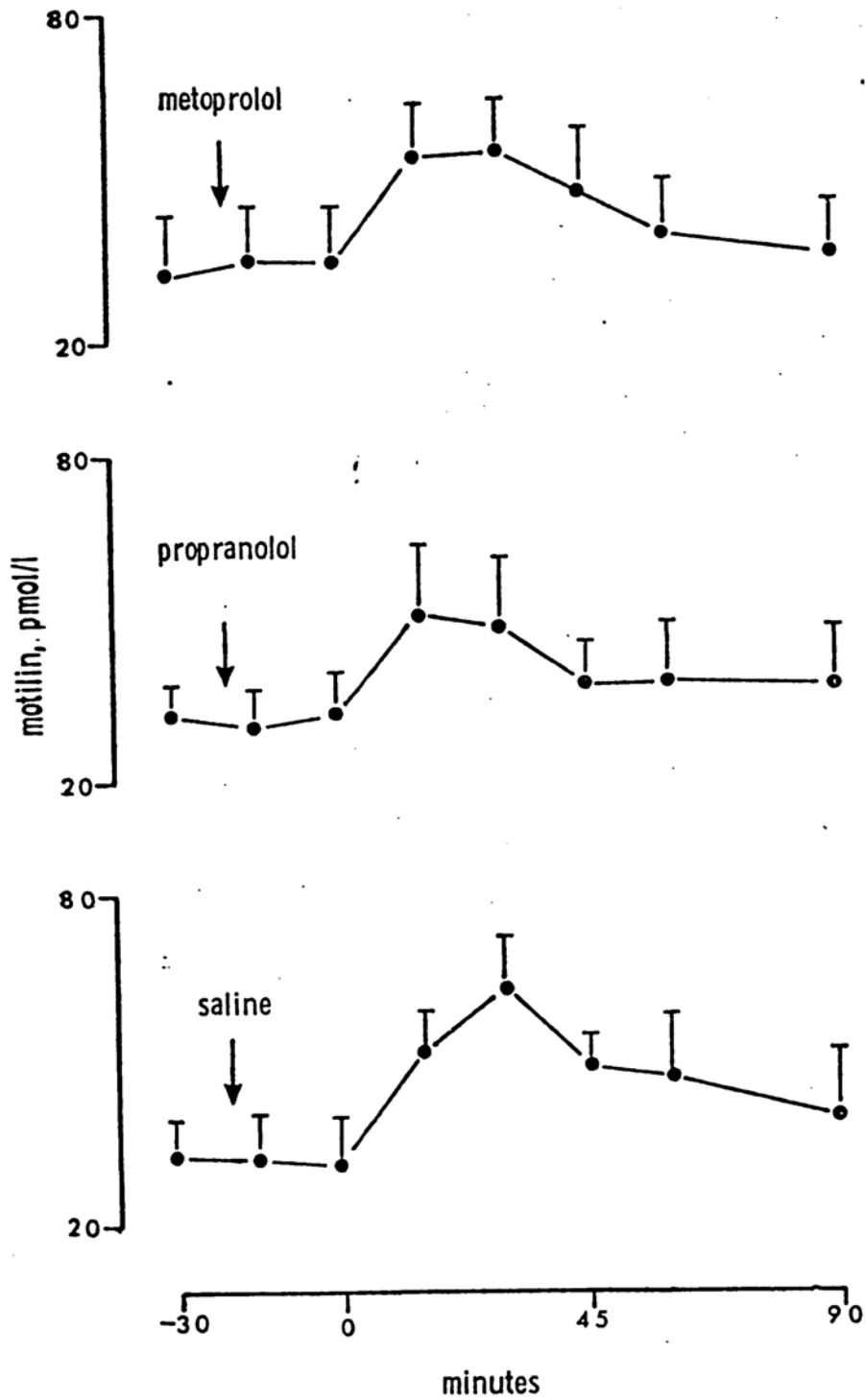


Figure 409

Plasma motilin (pmol/l) during antral balloon distension, following administration of saline (bottom), propranolol (middle) and metoprolol (top).

Intravenous stimulation

Nutrients

Figure 410 and Table 406 show the response of motilin to the intravenous infusions of the isocaloric nutrients. Infusions of glucose and Aminosol resulted in substantial falls of plasma motilin, the nadir achieved at 45 min (glucose 51 ± 9 pmol/l to 25 ± 6 pmol/l, Aminosol, 45 ± 10 pmol/l to 18 ± 6 pmol/l at zero and 45 min respectively). Infusion of Intralipid, however, resulted in an elevation of plasma motilin, which reached a peak at 30 min (49 ± 10 pmol/l to 74 ± 10 pmol/l).

Figure 411 and Table 407 show the effect of arginine given intravenously on plasma motilin. The motilin levels fell sharply reaching a nadir at 50 min (35 ± 5 pmol/l) from a basal value of $63 \pm$ pmol/l ($p < 0.001$)

Insulin

Figure 412 and Tables 408 and 409 show the effect of an insulin injection on plasma motilin levels. The upper graph shows the motilin response in the group that had their gastric juices drained and discarded. In both groups of subjects, insulin administration resulted in a significant suppression of the motilin values the nadir being at 30 min. Levels then gradually returned to pre-insulin values.

Pancreatic Polypeptide (PP)

Figure 413 and Tables 410 and 411 show the effect of PP on plasma motilin. Infusion of PP resulted in marked reduction in plasma motilin concentrations from 53 ± 13 pmol/l to 9 ± 5 pmol/l and 57 ± 12 to 13 ± 4 pmol/l (both $p < 0.001$) at the low and high doses respectively. The plasma motilin concentration quickly returned to basal on stopping the PP infusion. Infusion of saline alone had no significant effect of plasma motilin.

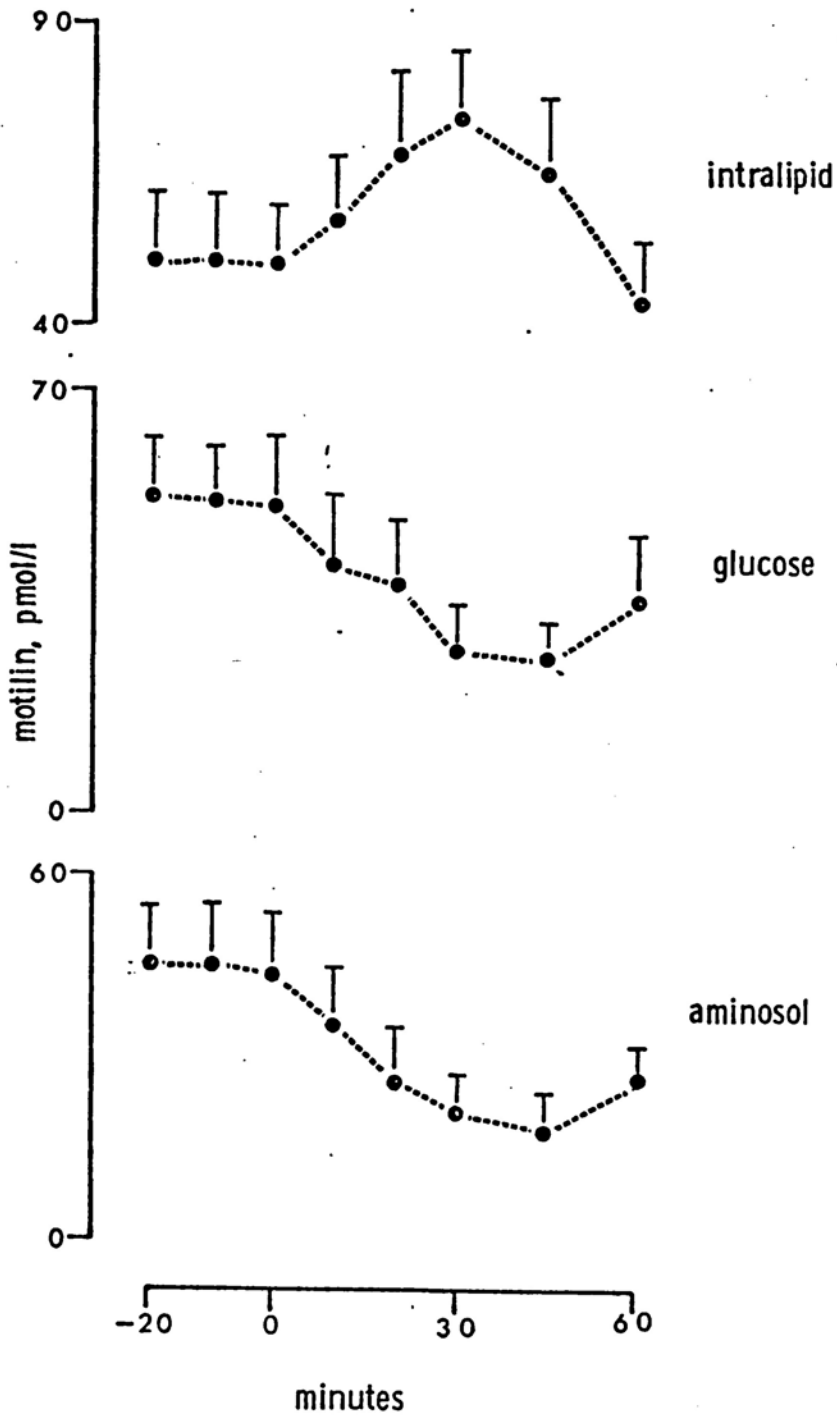


Figure 410

Plasma motilin (pmol/l) following intravenous infusion of mixture of amino-acids (bottom), glucose (middle) and Intralipid (top).

Sub	-20	-10	0	10	20	30	45	60
1	50	43	42	48	45	23	18	31
2	30	33	25	16	15	14	15	18
3	65	57	65	64	59	42	36	63
4	65	70	70	37	34	29	32	27
$\bar{X}$	52.5	50.5	50.5	41.2	38.2	27	25.2	34.8
SEM	9.6	9.3	12.1	11.6	10.7	6.8	6	11.3
1	39	38	40	50	52	63	66	56
2	30	32	34	34	43	59	45	28
3	56	60	47	67	83	75	53	25
4	75	75	75	77	93	100	95	63
$\bar{X}$	50	51	49	57	67.8	74.2	64.8	43
SEM	11.5	11.5	10.5	10.9	13.9	10.7	12.7	11.1
1	37	35	37	29	23	20	13	20
2	25	25	24	14	11	8	5	18
3	56	56	54	46	38	32	28	37
4	61	62	60	52	36	25	26	31
$\bar{X}$	45	45	44	35.3	27	21.2	18	26.5
SEM	9.7	10	9.5	9.9	7.3	5.8	6.3	5.2

Table 406

Plasma motilin (pmol/l) in 4 subjects receiving a 30 min intravenous infusion of aminoacids (bottom), Intralipid (middle) and glucose (top).

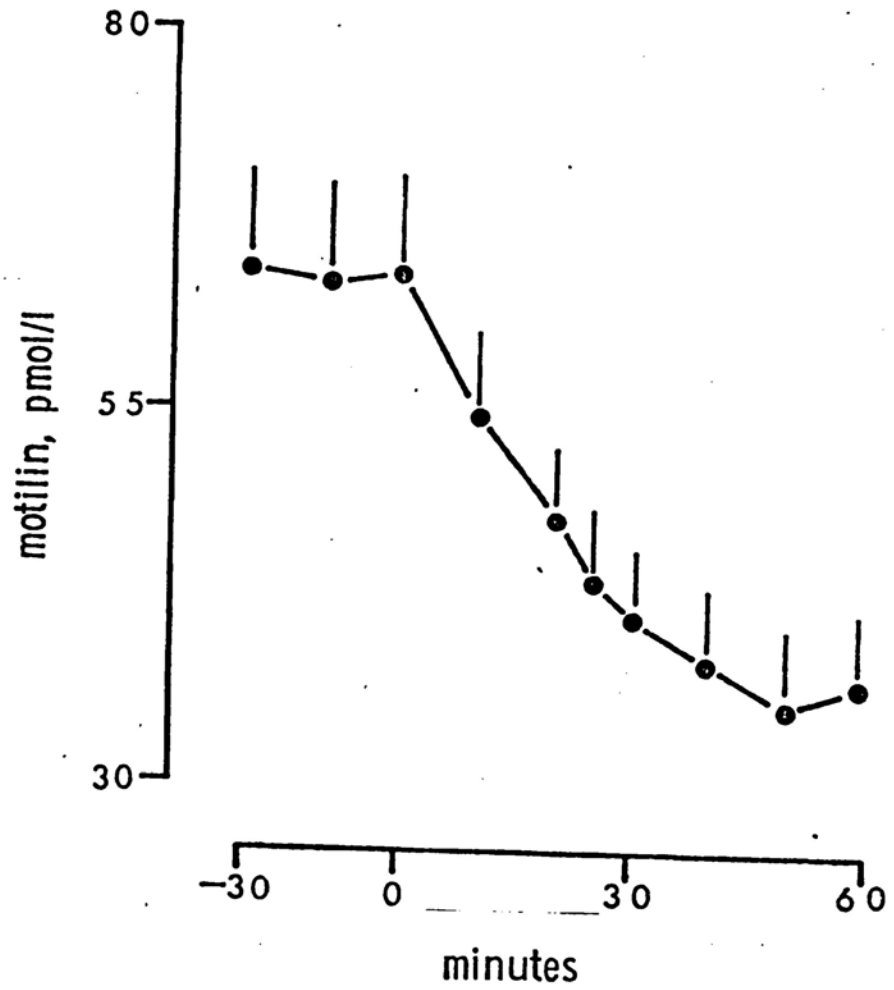


Figure 411

Plasma motilin (pmol/l) in 22 subjects receiving a 30 min intravenous infusion of arginine.

Sub	-20	-10	0	10	20	25	30	40	50	60
1	58	50	59	53	50	45	41	35	33	31
2	85	77	84	79	59	59	60	75	70	47
3	147	145	143	115	102	90	88	94	84	81
4	100	104	97	88	87	87	87	90	88	100
5	44	44	49	41	35	27	26	22	20	16
6	77	72	76	63	51	39	49	51	36	35
7	86	87	84	36	30	28	22	20	22	20
8	41	44	46	44	41	42	32	29	25	36
9	32	38	34	39	29	21	21	20	13	10
10	30	30	28	23	24	20	19	16	15	16
11	60	62	20	56	36	32	33	24	36	35
12	64	63	60	53	45	43	43	36	32	31
13	47	47	47	37	34	35	33	30	32	30
14	90	91	90	85	67	63	62	57	53	48
15	20	20	20	17	15	14	13	16	12	21
16	35	29	34	18	16	12	9	9	10	12
17	75	78	76	77	75	65	59	58	55	50
18	30	29	28	25	26	24	21	15	12	16
19	55	55	60	62	51	44	38	33	32	32
20	104	104	103	90	85	81	70	56	58	69
21	57	54	56	48	43	39	33	29	25	33
22	67	66	67	51	47	44	39	37	38	33
$\bar{X}$	63.8	63.1	63.7	54.5	47.6	43.4	40.8	37.9	35.5	36.5
SEM	6.6	6.6	6.4	5.6	5.1	4.9	4.8	5	4.7	4.9
Log transformation of raw data										
X	1.76	1.75	1.76	1.69	1.63	1.58	1.55	1.51	1.47	1.49
SEM	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06

Table 407

Plasma motilin (pmol/l) in 22 subjects receiving a 30 min intravenous infusion of arginine (0.5 g/Kg/hour)

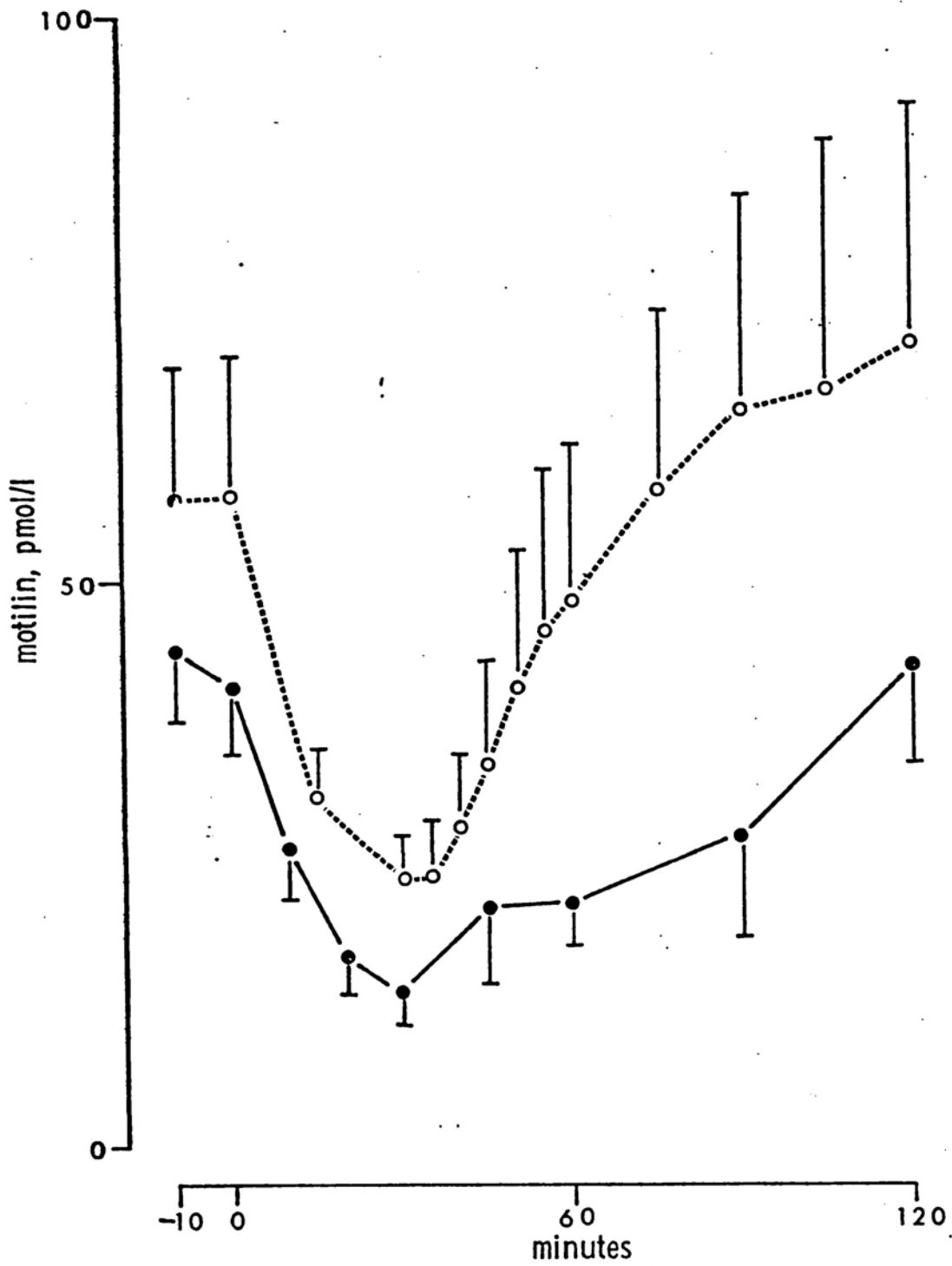


Figure 412

Plasma motilin following insulin administration. Solid line (gastric acid was drained) and broken line (gastric acid was not drained)

Sub	-10	0	15	30	35	40	45	50	55	60	75	90	105	120
1	60	60	28	13	18	24	46	56	57	52	74	78	69	74
2	46	40	26	17	14	15	12	18	16	20	20	16	11	15
3	36	36	16	28	22	32	29	24	29	31	80	100	90	108
4	108	111	46	26	32	28	32	48	69	82	81	89	92	92
5	48	52	27	20	12	14	12	12	10	11	6	7	8	6
6	44	48	40	36	42	54	68	84	92	90	88	101	132	130
X	57	57.8	30.5	23.3	23.3	27.8	33.2	40.3	45.5	47.7	58.2	65.2	67	70.8
SEM	11.7	12.3	4.8	3.7	5.2	6.6	9.6	12.3	14.5	14.7	15.9	19	21.9	22.5
<u>log transformation of raw data</u>														
X	1.726	1.73	1.46	1.344	1.325	1.40	1.437	1.51	1.54	1.57	1.62	1.65	1.637	1.656
SEM	0.07	0.08	0.07	0.07	0.09	0.1	0.14	0.14	0.17	0.16	0.21	0.22	0.23	0.24

Table 408

Plasma motilin following insulin administration (gastric acid was not drained).

Sub	-10	0	10	20	30	45	60	90	120
1	41	36	18	18	11	66	29	73	92
2	23	30	25	14	20	28	34	55	27
3	22	20	11	5	8	8	8	8	26
4	65	66	45	34	23	20	32	58	41
5	26	21	12	6	2	6	25	12	28
6	47	50	38	20	8	9	6	14	21
7	54	47	31	18	16	15	10	44	54
8	62	54	30	17	18	17	28	34	50
X	43.7	40.5	26.3	16.5	13.3	21.1	21.5	27.3	42.4
SEM	6.1	6.2	4.6	3.4	2.7	7.4	4.4	9.2	8.8
<u>log transformation of raw data</u>									
X	1.61	1.57	1.37	1.15	1.03	1.2	1.25	1.46	1.58
SEM	0.07	0.07	0.09	0.01	0.13	0.13	0.12	0.14	0.08

Figure 409

Plasma motilin (pmol/l) following insulin administration (the gastric acid was drained).

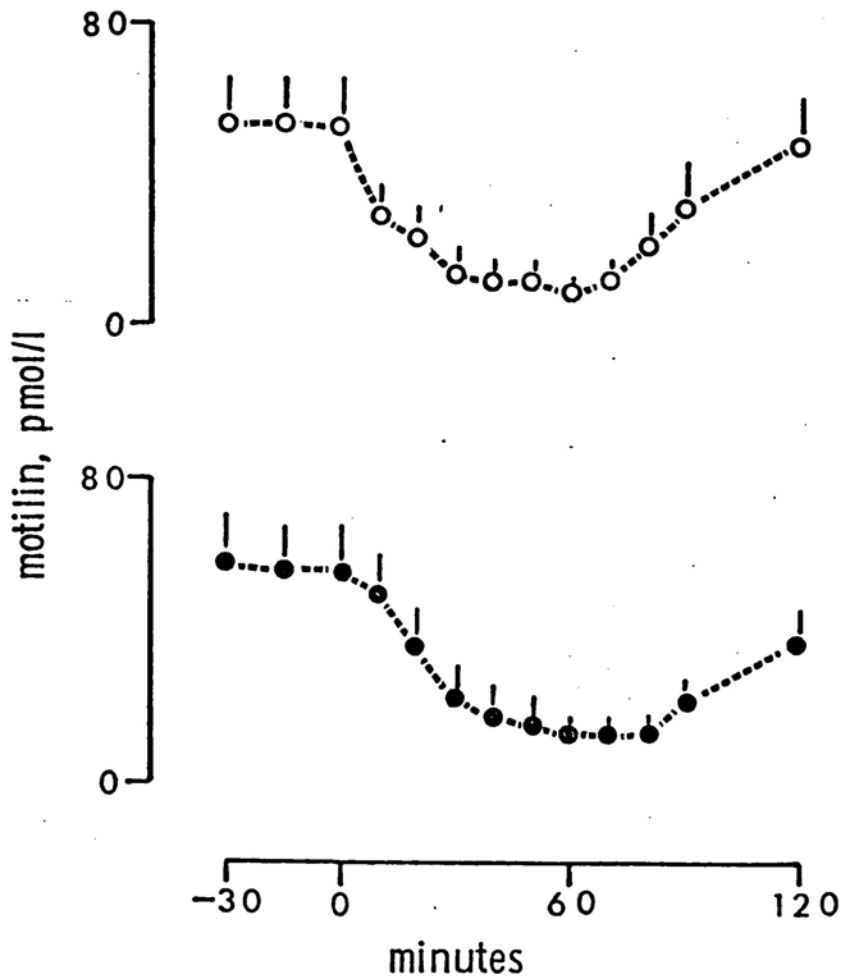


Figure 413

Plasma motilin (pmol/l) following a 60 min intravenous infusion of a low dose (top) and high dose (bottom) of bovine pancreatic polypeptide.

Sub	-30	-15	0	10	20	30	40	50	60	70	80	90	120
1	49	45	49	8	6	2	2	2	2	6	13	29	44
2	23	26	25	20	8	5	3	2	2	2	8	14	22
3	95	96	98	29	31	33	26	29	20	28	45	65	87
4	27	25	28	16	14	8	6	2	3	6	12	19	25
5	15	14	15	8	2	2	2	3	2	3	6	9	13
6	74	74	72	38	30	8	3	4	3	2	6	22	48
7	88	90	86	80	69	44	40	38	30	40	60	76	96
$\bar{X}$	53	52.9	53.3	28.4	22.9	14.6	11.7	11.4	8.9	12.4	21.4	33.4	47.9
SEM	13.4	13.7	13.3	10.3	9.5	6.9	6.2	6.2	4.7	6.2	8.9	10.7	13.2
<u>log transformation of raw data</u>													
X	1.64	1.63	1.64	1.32	1.13	0.90	0.76	0.72	0.66	0.81	1.15	1.41	1.59
SEM	0.13	0.13	0.13	0.15	0.21	0.22	0.22	0.23	0.21	0.22	0.17	0.14	0.13

Table 410

Plasma motilin (pmol/l) in 7 subjects receiving a 60 min intravenous infusion of bovine pancreatic polypeptide (1.19 ± 0.15 pmol/Kg/min).

Sub	-30	-15	0	10	20	30	40	50	60	70	80	90	120
1	10	9	9	7	6	3	2	4	5	4	2	4	8
2	28	31	38	30	21	9	6	4	2	3	4	2	16
3	32	32	30	24	6	5	4	5	2	3	3	4	10
4	76	62	72	61	59	31	19	17	14	13	9	14	38
5	125	106	111	106	77	56	57	48	31	36	43	63	100
6	56	55	50	43	30	22	15	12	7	6	8	32	50
7	47	47	58	48	36	31	23	22	22	15	17	23	35
8	130	136	123	115	91	64	53	46	35	28	26	30	55
9	43	49	50	41	27	20	15	14	14	17	17	21	37
10	25	28	25	19	6	2	2	2	2	8	12	19	27
$\bar{X}$	57.2	55.5	56.6	49.4	35.9	24.3	19.6	17.4	13.4	13.3	14.1	21.2	37.6
SEM	13.8	12.8	12.2	11.9	10.1	7.2	6.7	5.6	4.1	3.7	4.2	6.1	9
<u>log transformation of raw data</u>													
X	1.65	1.65	1.656	1.58	1.37	1.16	1.05	1.03	0.91	0.98	0.98	1.14	1.47
SEM	0.11	0.11	0.11	0.12	0.15	0.18	0.18	0.16	0.16	0.13	0.14	0.16	0.11

Table 411

Plasma motilin (pmol/l) in 10 subjects receiving a 60 mins intravenous infusion of bovine pancreatic polypeptide (4.2 ± 0.38 pmol/Kg/min).

Bombesin

The effect of bombesin on plasma motilin is shown in figure 414 and tables 412 and 413. When bombesin was infused at the low dose of 0.3 pmol/Kg/min no significant alteration in motilin concentration occurred. However, when bombesin was infused at 1.2 pmol/Kg/min a significant release of motilin resulted. Plasma motilin levels started to rise in the latter half of the infusion, the peak rise being achieved 15 min following the cessation of the bombesin infusion ($p < 0.005$).

Somatostatin

There was a significant fall of motilin following the somatostatin infusion (Figure 415) with levels being suppressed to a concentration of only 6 ± 2 pmol/l from a basal of 28 ± 4 pmol/l ($p < 0.001$).

Atropine

Injection of atropine resulted in a significant ($p < 0.001$) suppression of motilin (Figure 416 and Table 414). This suppression was maintained for 3 hours.

Motilin during pregnancy and early life

Figure 417 and Tables 415, 416 and 417 show the plasma motilin concentrations in 19 normal weight women at 16 and 36 weeks of pregnancy and at 1 week postpartum. The mean fasting motilin level was 24 ± 3 pmol/l and 20 ± 3 pmol/l at 16 and 36 weeks of pregnancy. These values were significantly lower than the corresponding value at 1 week postpartum (50 ± 6 pmol/l, both $p < 0.001$). Following ingestion of the glucose, motilin levels fell to 16 ± 2 pmol/l, 12 ± 2 pmol/l and 27 ± 3 pmol/l at 16 and 36 weeks of pregnancy and at 1 week postpartum respectively (all $p < 0.005$). Following ingestion of the meal motilin rose in all 3 groups (at 16 weeks, 16 ± 3 pmol/l to 26 ± 3 pmol/l, $p < 0.001$), at 36 weeks, 12 ± 2 pmol/l to 27 ± 3 pmol/l ($p < 0.001$) and at 1 week postpartum from 27 ± 3 pmol/l to 51 ± 4 pmol/l ($p < 0.001$).

The difference between the fasting motilin levels at 16 weeks was not statistically different from that at 36 weeks of pregnancy.

Sub	-30	-15	0	5	10	15	20	25	30	35	40	45	60	75
1	37	23	19	6	14	14	14	19	30	33	43	46	59	46
2	22	16	16	10	12	18	12	28	38	26	32	31	39	41
3	53	49	61	43	40	36	41	35	50	54	46	68	71	61
4	33	48	64	54	49	29	30	31	42	49	56	66	88	58
5	10	8	11	11	8	9	10	24	24	29	33	38	28	28
6	68	60	63	56	58	61	90	91	82	98	112	126	110	70
X	37.2	34	39	30	30.2	27.8	32.8	38	44.3	48.2	54.7	62.5	65.8	50.7
SEM	9.4	9.4	11.7	10.5	9.6	8.5	13.6	11.9	9.2	12	13.6	15.4	13.7	6.8
Log transformation of raw data														
X	1.5	1.44	1.486	1.32	1.364	1.36	1.38	1.52	1.61	1.635	1.7	1.75	1.77	1.69
SEM	0.13	0.15	0.16	0.19	0.16	0.13	0.17	0.11	0.08	0.1	0.09	0.1	0.1	0.06

Table 412

Plasma motilin (pmol/l) following intravenous bombesin (1.2pmol/Kg/min).

Sub	-30	-15	0	5	10	15	20	25	30	35	40	45	60	75
1	32	30	32	30	17	20	18	15	17	22	30	46	48	41
2	17	23	27	30	43	58	37	39	66	50	60	44	41	43
3	70	68	60	65	48	46	37	45	41	57	62	78	71	67
4	72	62	61	60	64	67	58	60	40	56	62	55	71	66
5	43	41	43	30	34	30	20	24	26	37	29	35	30	35
6	42	58	81	90	70	65	62	71	80	95	86	79	94	66
X	46	47	50.7	50.8	46	47.7	38.7	42.3	45	52.8	54.8	56.2	59.1	53
SEM	9.6	8.2	9.1	11.2	8.7	8.6	8.2	9.5	10.7	11	9.8	8.2	10.6	6.6
<u>log transformation of raw data</u>														
X	1.62	1.64	1.67	1.66	1.62	1.64	1.54	1.57	1.60	1.68	1.71	1.73	1.74	1.71
SEM	0.1	0.08	0.08	0.09	0.1	0.09	0.1	0.11	0.11	0.1	0.09	0.06	0.08	0.06

Table 413

Plasma motilin (pmol/l) following intravenous bombesin (0.3pmol/Kg/min).

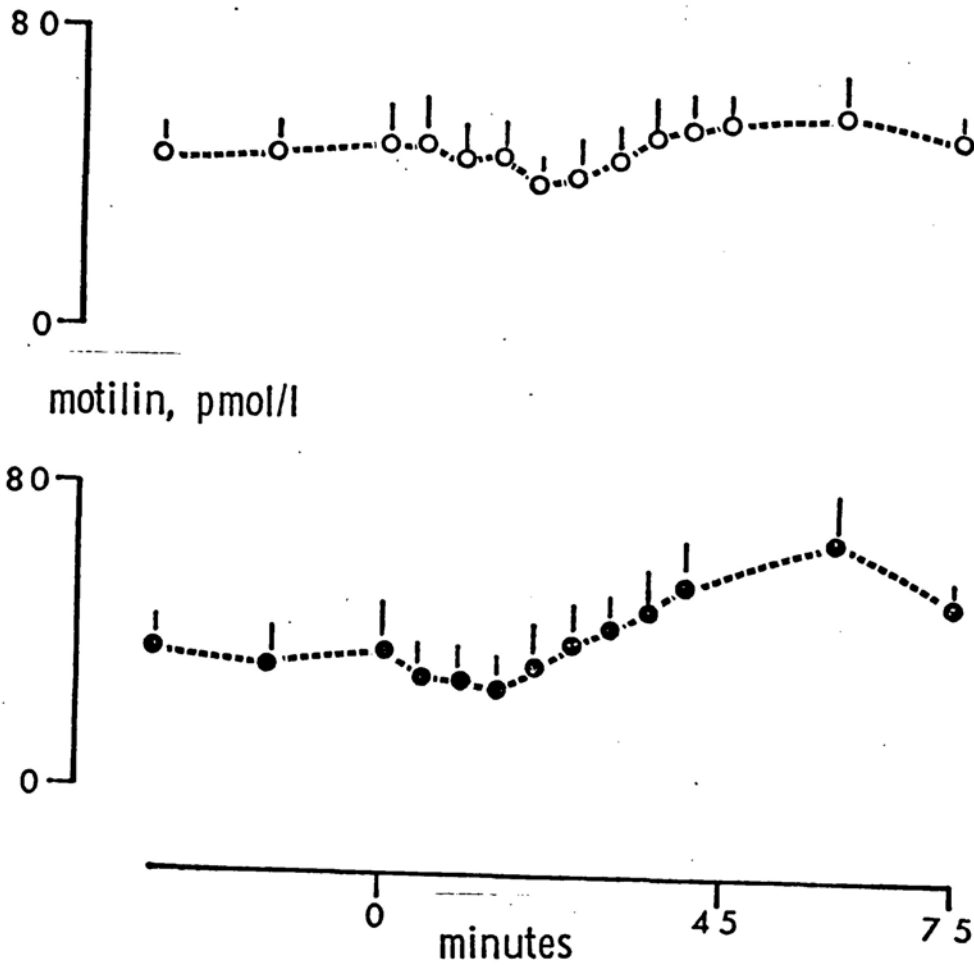


Figure 414

Plasma motilin following infusion of bombesin 0.3 pmol/Kg/min (top) and 1.2 pmol/Kg/min (bottom).

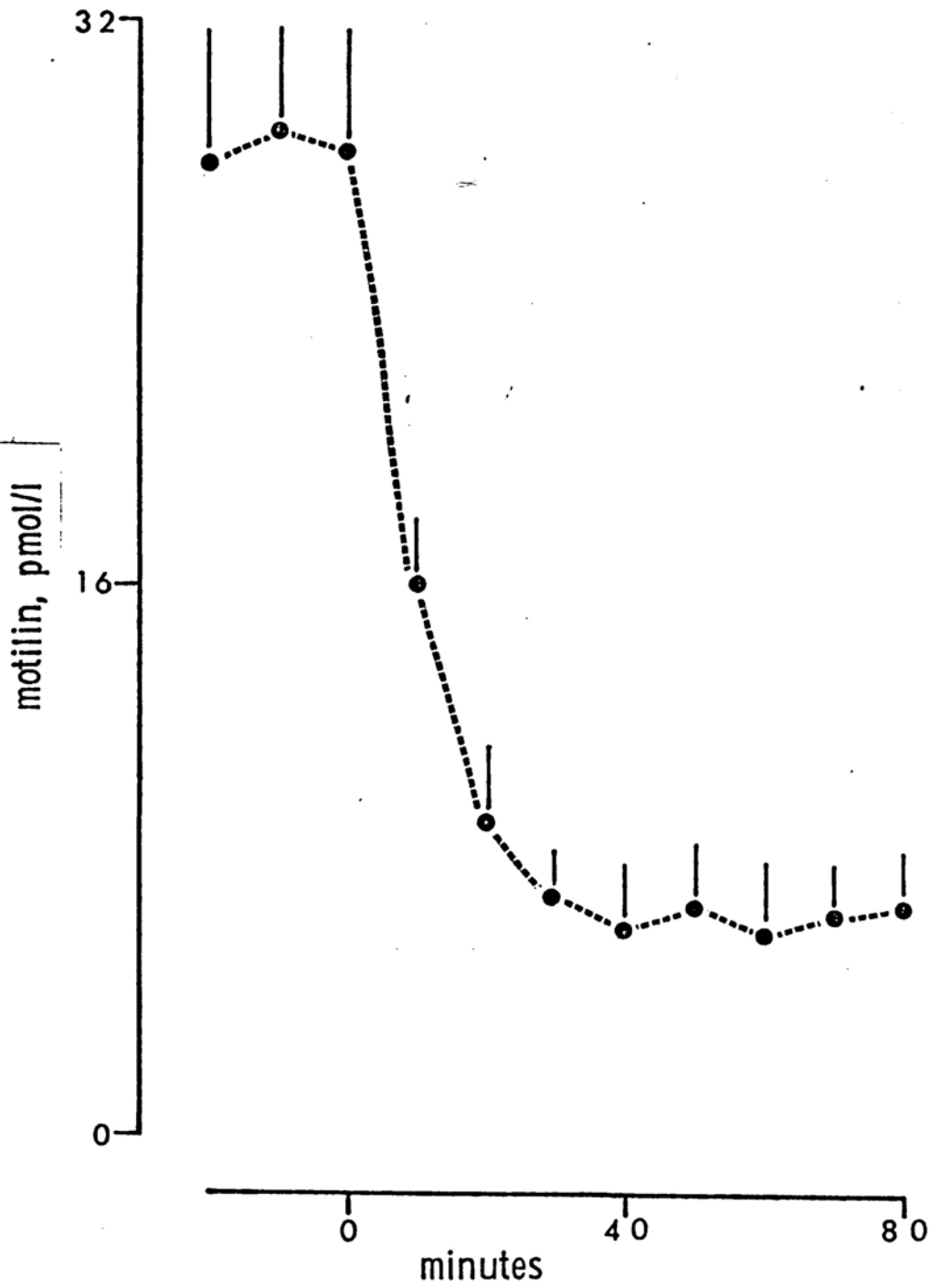


Figure 415.

Plasma motilin following infusion of somatostatin

Sub	-30	0	15	30	45	60	90	120	150	180	210	240
1	56	52	34	20	30	23	25	18	26	49	82	40
2	25	25	15	16	13	9	3	4	4	8	15	25
3	52	48	32	21	22	16	24	17	35	22	20	36
4	50	50	42	26	16	19	16	22	11	21	21	18
5	22	22	18	21	14	8	6	8	2	6	16	24
6	55	52	52	40	33	29	45	35	44	46	55	59
$\bar{X}$	43.3	41.5	32.2	24	21.3	17.3	19.8	17.3	20.3	25.3	34.8	33.7
SEM	7	6.3	6.3	3.8	3.8	3.6	6.8	4.9	7.7	8.2	12.3	6.6
<u>Log transformation of raw data</u>												
X	1.61	1.59	1.47	1.36	1.3	1.19	1.15	1.15	1.09	1.28	1.44	1.49
SEM	0.08	0.08	0.09	0.05	0.08	0.1	0.2	0.15	0.24	0.17	0.14	0.08

Table 414

Plasma motilin (pmol/l) in 6 subjects receiving an intravenous injection of 600 ug atropine (at time 0).

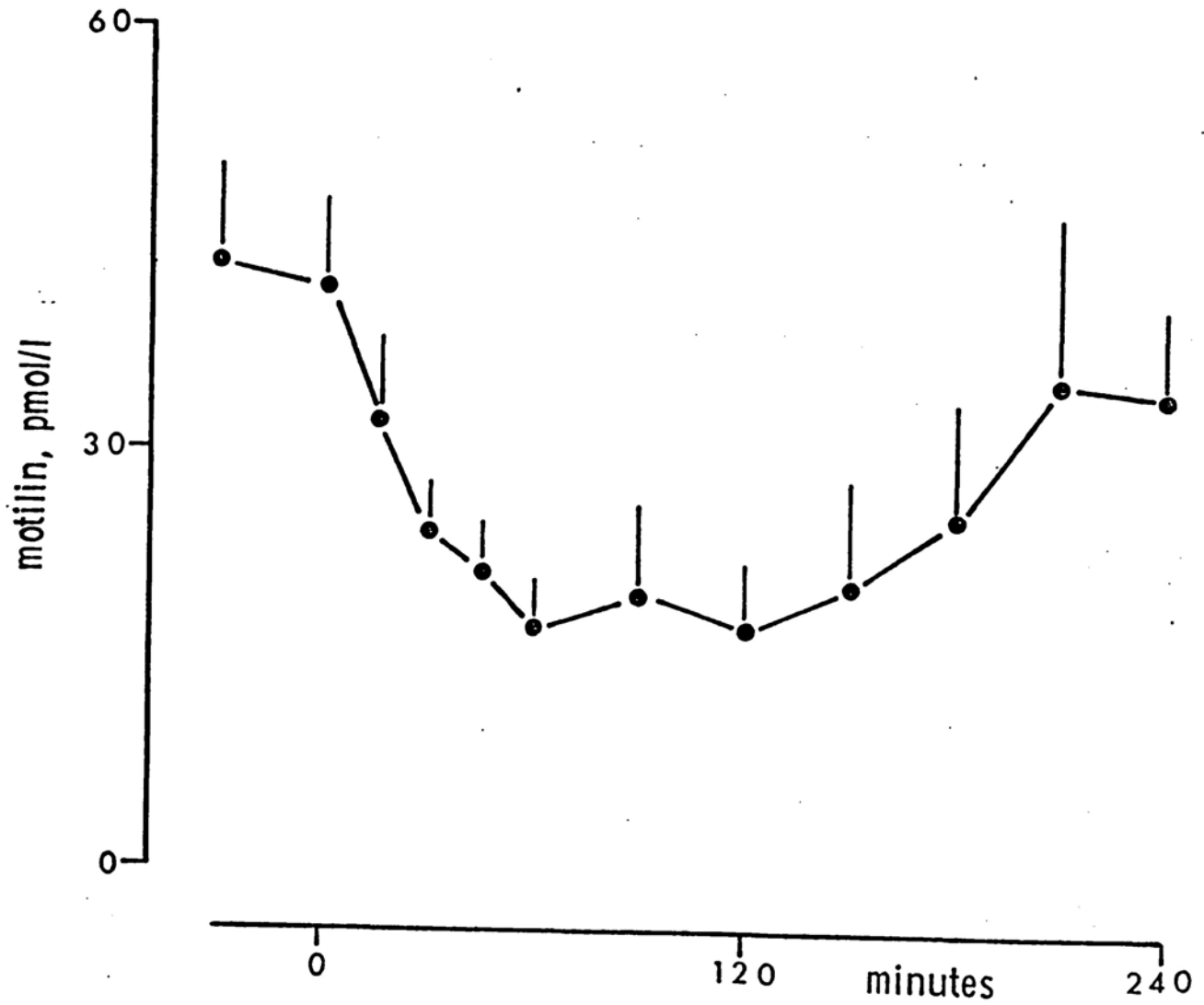


Figure 416

Plasma motilin following atropinisation.

Sub	-30	0	30	60	90	120	150	180	210	240	270	300	330	360
1	10	13	8	9	8	10	3	20	15	4	8	2	8	14
2	27	27	40	40	22	20	29	13	36	46	50	49	51	49
3	28	35	28	39	18	19	26	25	27	40	26	33	22	28
4	11	16	19	7	9	8	9	9	10	9	10	9	14	15
5	21	24	38	24	16	17	7	14	21	15	22	14	12	17
6	42	44	50	42	42	36	30	34	47	38	30	40	27	39
7	13	18	13	20	17	21	34	33	42	60	60	29	50	61
8	54	51	64	48	36	46	53	47	54	49	44	46	62	38
9	24	18	21	13	12	10	9	8	30	26	44	36	38	54
10	12	7	10	10	12	12	10	6	16	12	19	22	36	17
11	27	30	35	14	22	14	11	10	20	21	6	16	7	18
12	16	15	18	5	6	8	8	12	30	25	8	16	21	7
13	31	24	36	16	12	4	15	13	27	26	25	20	11	8
14	14	14	19	11	12	15	6	9	24	20	32	22	26	20
15	38	37	10	13	7	7	7	8	12	27	21	20	18	18
16	27	25	14	26	24	18	12	9	14	23	16	19	32	25
17	6	5	12	4	3	5	9	2	19	11	14	4	3	4
18	16	20	7	13	11	14	14	17	26	15	10	20	9	5
19	33	30	32	33	10	14	9	16	19	40	34	32	16	30
X	23.7	23.8	24.9	20.3	15.7	15.7	15.8	16	25.7	26.7	25.2	23.6	24.4	24.6
SEM	2.9	2.8	3.7	3.2	2.4	2.4	3	2.7	2.8	3.6	3.7	3.1	3.9	3.9
<u>log transformation of raw data</u>														
X	1.31	1.32	1.31	1.21	1.12	1.12	1.09	1.11	1.37	1.35	1.31	1.28	1.28	1.28
SEM	0.06	0.006	0.07	0.07	0.06	0.06	0.07	0.07	0.05	0.07	0.07	0.08	0.08	0.08

Table 415

Plasma motilin (pmol/l) in 19 women (normal weight) at 16 weeks of pregnancy. The glucose was given at time 0 and the meal at time 180.

Sub	-30	0	30	60	90	120	150	180	210	240	270	300	330	360
1	4	5	10	4	2	2	4	4	16	5	6	7	5	8
2	9	9	7	5	12	9	9	8	49	43	55	51	71	82
3	10	16	12	18	14	14	12	10	23	30	17	14	20	42
4	18	14	4	3	3	2	6	5	21	16	18	10	6	12
5	7	6	11	12	9	7	9	6	14	9	18	8	8	10
6	17	27	26	17	22	5	14	10	19	21	18	25	8	18
7	32	28	28	29	27	24	30	34	52	60	38	32	44	44
8	62	54	47	34	43	40	31	38	52	56	53	52	32	38
9	14	13	15	18	11	11	4	2	30	17	12	12	20	13
10	25	25	30	23	10	22	14	12	36	46	42	42	53	26
11	33	31	28	28	26	8	10	18	26	26	14	28	28	37
12	10	16	26	11	5	2	7	11	16	16	18	23	32	19
13	15	23	25	12	13	14	14	8	35	33	38	36	26	24
14	7	9	14	14	11	14	13	7	22	15	23	18	26	24
15	18	16	18	15	22	9	12	12	20	18	30	28	20	32
16	30	39	37	33	16	8	16	15	22	13	30	32	36	44
17	10	9	7	3	10	6	10	6	20	14	8	8	23	14
18	15	15	12	9	8	11	6	12	24	16	13	16	16	23
19	21	24	28	29	20	20	12	13	22	20	31	23	26	30
X	18.8	19.9	20.8	16.7	14.9	12	12.3	12.2	27.3	24.9	25.4	24.5	26.3	28.4
SEM	3.2	2.9	3	2.4	2.7	2.2	1.7	2.2	2.8	3.7	3.4	3.3	3.9	4.1
<u>log transformation of raw data</u>														
X	1.18	1.22	1.23	1.12	1.07	0.95	0.99	0.99	1.40	1.32	1.33	1.32	1.33	1.38
SEM	0.07	0.06	0.07	0.08	0.08	0.09	0.07	0.07	0.04	0.06	0.06	0.06	0.07	0.06

Table 416

Plasma motilin (pmol/l) in 19 women (normal weight) at 36 weeks of pregnancy. The glucose was given at time 0 and the meal at time 180.

Sub	-30	0	30	60	90	120	150	180	210	240	270	300	330	360
1	37	45	26	23	18	16	7	6	36	25	15	13	14	14
2	75	80	86	76	32	22	43	58	110	82	89	90	104	64
3	47	44	47	30	32	44	40	32	70	58	76	42	75	28
4	96	100	57	35	20	34	43	39	54	76	54	40	42	40
5	22	26	30	26	17	18	21	10	26	46	18	26	28	22
6	27	36	35	32	26	28	31	25	40	36	42	41	42	28
7	23	27	12	11	9	6	24	20	42	42	37	38	36	38
8	70	67	68	53	52	50	44	49	65	60	84	86	98	115
9	24	30	26	14	13	14	16	13	24	16	18	10	22	9
10	33	33	34	26	24	21	36	26	53	46	51	36	34	60
11	98	110	77	55	54	46	42	36	68	31	16	21	56	36
12	48	36	40	40	28	42	38	34						
13	36	46	38	36	36	38	36	36	53	45	30	52	38	48
14	46	45	46	44	44	38	29	29	48	46	47	32	52	74
15	39	48	40	40	16	25	13	13	38	42	74	54	54	64
16	68	71	58	66	58	58	36	24	74	66	65	66	60	58
17	26	20	26	11	14	6	8	9	30	26	23	14	19	28
18	28	26	30	26	22	26	22	17						
19	57	66	33	17	35	31	27	30	43	43	58	55	64	43
$\bar{X}$	47.4	50.3	42.6	34.8	28.9	29.6	29.3	26.6	51.4	46.2	46.8	42.1	46.9	45.2
SEM	5.6	6	4.5	4.2	3.4	3.5	2.8	3.3	5.3	4.4	6.2	5.8	5.6	6.5
<u>log transformation of raw data</u>														
X	1.63	1.65	1.59	1.48	1.41	1.40	1.415	1.36	1.68	1.63	1.60	1.55	1.62	1.58
SEM	0.05	0.05	0.05	0.06	0.05	0.07	0.06	0.06	0.04	0.05	0.07	0.07	0.06	0.07

Table 417

Plasma motilin (pmol/l) in 19 women (normal weight) at 1 week post-partum.
The glucose was given at time 0 and the meal at time 180.

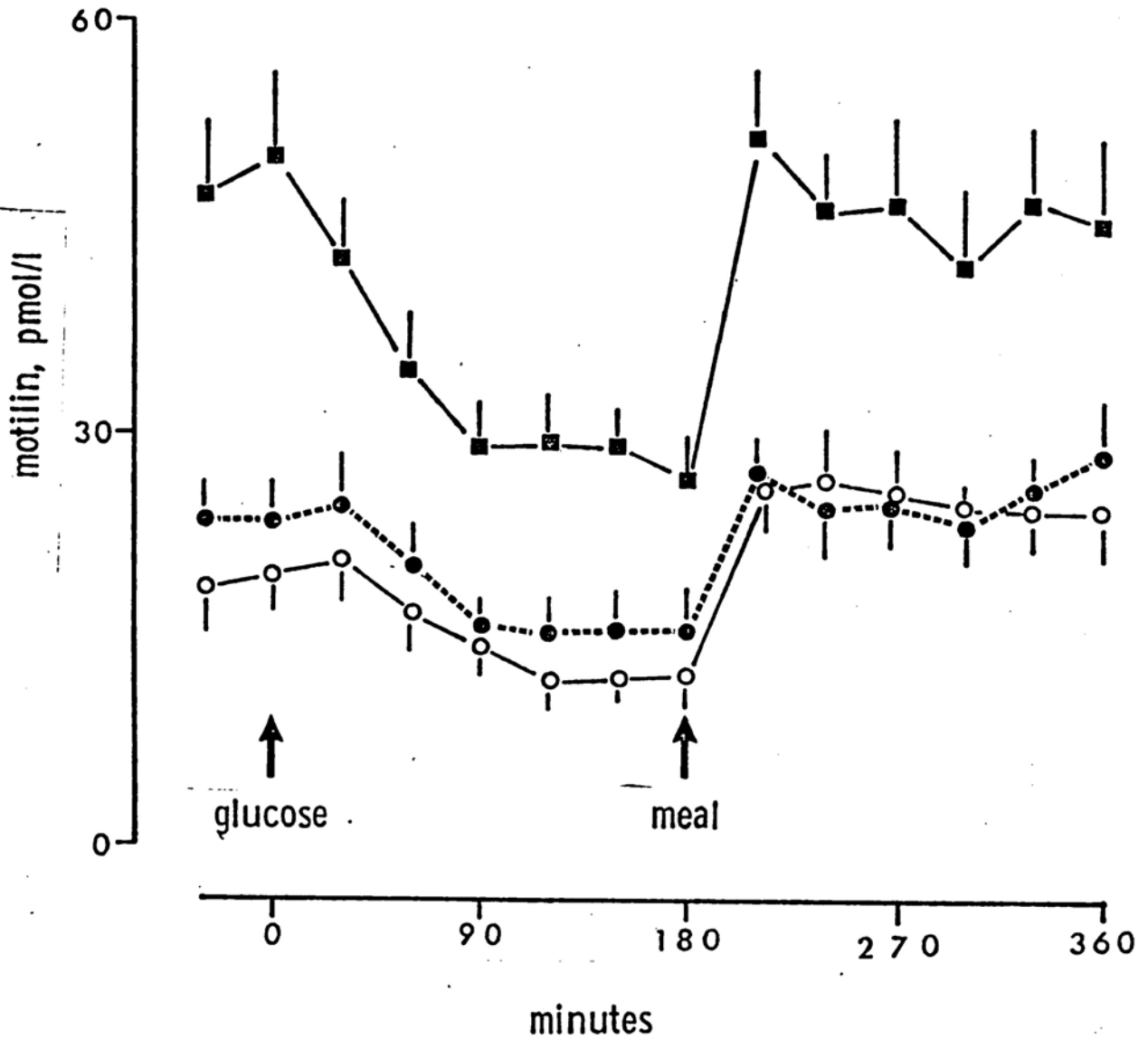


Figure 417

Plasma motilin in 19 normal weight pregnant women. Solid squares depict levels at 1 week post-partum, open circles at 36 weeks pregnancy and solid circles at 16 weeks pregnancy.

The postprandial release of motilin at 1 week postpartum ($24.6 \pm 3 \text{ pmol/l}$) was significantly higher than that seen at 16 weeks ($9.2 \pm 1.8 \text{ pmol/l}$, $p < 0.001$) and at 36 weeks ($16 \pm 2 \text{ pmol/l}$, $p < 0.002$). The release at 16 weeks was also significantly lower ($p < 0.002$) than that seen at 36 weeks although there was no difference in the fasting motilin concentration: (In the obese subjects).

Figure 418 and Tables 418, 419 and 420 show the plasma motilin concentrations at 16 and 36 weeks and at 1 week postpartum. The fasting motilin levels at 16 weeks ($27 \pm 5 \text{ pmol/l}$) and 36 weeks ($23 \pm 4 \text{ pmol/l}$) were significantly lower ($p < 0.001$) than those at 1 week postpartum ($67 \pm 8 \text{ pmol/l}$). Following ingestion of glucose the motilin values fell to $16 \pm 3 \text{ pmol/l}$, $17 \pm 3 \text{ pmol/l}$ and $41 \pm 6 \text{ pmol/l}$ at 16 and 36 weeks of pregnancy and at 1 week postpartum, respectively.

Plasma motilin rose significantly ($p < 0.001$), following ingestion of the meal, in all three groups (at 16 weeks $16 \pm 3 \text{ pmol/l}$ to $32 \pm 4.5 \text{ pmol/l}$, at 36 weeks $19 \pm 3 \text{ pmol/l}$ to $37.5 \pm 5 \text{ pmol/l}$, and at 1 week postpartum $41 \pm 6 \text{ pmol/l}$ to $72 \pm 9.5 \text{ pmol/l}$). The postprandial rise at 1 week postpartum ($30.2 \pm 5 \text{ pmol/l}$) was significantly higher than the corresponding rises at 16 weeks ($15.4 \pm 2 \text{ pmol/l}$ $p < 0.02$) and 36 weeks ($18.6 \pm 3.5 \text{ pmol/l}$ $p < 0.005$). The postprandial release at 16 weeks was not significantly different from that seen at 36 weeks.

Mean cord plasma motilin concentration in 20 term infants was 32 ± 4 (mean $\pm$ SEM) pmol/l , significantly higher than in 6 preterm infants 13 ± 4 ($p < 0.02$). No significant change had occurred in plasma levels by 3-6 hours of age before the first feed in either term (26 ± 3) or preterm (14 ± 4) infants.

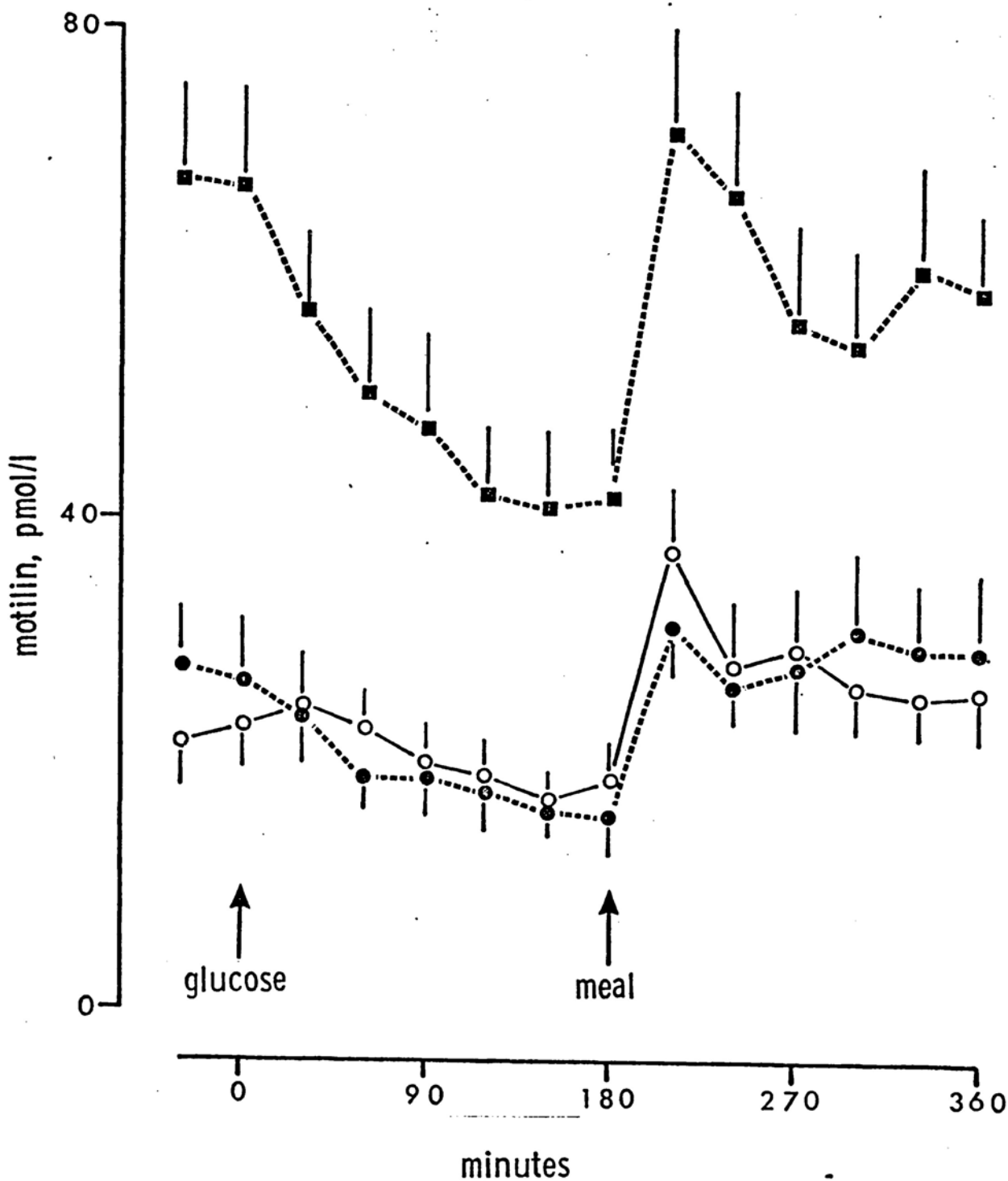


Figure 418

Plasma motilin in 18 obese pregnant women.. Solid squares depict levels at 1 week post-partum, open circles at 36 weeks pregnancy and solid circles at 6 weeks pregnancy.

Sub	-30	0	30	60	90	120	150	180	210	240	270	300	330	360
1	93	90	95	68	60	67	32	33	36	48	38	62	53	90
2	12	12	9	10	6	8	10	4	12	9	6	7	10	6
3	14	12	11	6	8	7	3	4	15	15	10	13	7	5
4	30	23	14	19	15	10	4	14	40	16	14	18	16	26
5	13	10	12	10	10	8	14	7	18	20	16	8	24	12
6	19	19	8	9	4	4	5	6						
7	20	18	18	13	16	18	16	6	22	20	21	24	19	24
8	15	13	10	12	13	10	14	12	15	11	15	14	9	13
9	17	16	15	14	10	6	14	5	25	18	14	14	26	22
10	7	8	4	6	7	7	7	4	15	16	3	8	8	8
11	45	48	31	35	36	30	24	34	50	46	37	30		
12	17	19	14	16	19	26	15	16	22	20	18	15	25	26
13	44	45	36	24	27	28	22	28	54	35	50	74	30	12
14	20	19	20	15	19	14	17	10	30	27	25	26	36	29
15	40	45	46	36	32	32	37	39	66	59	78	83	70	62
16	30	28	30	10	26	18	23	20	42	32	58	57	40	54
17	18	19	15	6	6	9	10	3	12	15	16	12	7	3
18	49	42	42	37	34	32	32	37	63	44	57	62	86	74
$\bar{X}$	27.9	27	23.9	19.2	19.3	18	16.6	15.7	31.6	26.5	28	31	29.1	29.1
SEM	5	4.9	5.2	3.9	3.5	3.7	2.4	3.1	4.5	3.7	5.4	6.4	6	6.9
<u>log transformation of raw data</u>														
$\bar{X}$	1.36	1.34	1.25	1.176	1.176	1.136	1.13	1.04	1.43	1.36	1.32	1.35	1.335	1.28
SEM	0.07	0.07	0.08	0.07	0.08	0.08	0.08	0.09	0.06	0.06	0.09	0.09	0.09	0.11

Table 418

Plasma motilin (pmol/l) in 18 obese women at 16 weeks of pregnancy.
The glucose was given at time 0 and the meal at time 180.

Sub	-30	0	30	60	90	120	150	180	210	240	270	300	330	360
1	40	46	48	46	48	43	35	55	66	80	55	58	58	76
2	6	6	3	6	11	11	6	7	8	6	6	11	6	10
3	7	7	16	17	12	9	8	7	12	14	14	13	13	15
4	6	4	6	8	8	2	2	3	16	7	4	3	12	10
5	3	5	5	8	6	9	13	20	32	13	20	12	16	16
6	18	20	19	22	10	14	6	4	48	18	30	13	21	7
7	9	6	14	16	11	10	10	4	32	32	25	17	22	25
8	20	23	32	21	18	12	18	10	35	22	19	28	20	22
9	30	34	20	32	28	31	28	33	45	47	50	16	30	22
10	11	7	7	4	4	9	16	23	37	22	32	14	20	18
11	16	24	26	28	8	8	8	13	28	23	16	26	16	27
12	18	16	24	25	18	14	16	12	14	11	10	14	21	24
13	42	40	22	28	24	26	14	27	65	40	51	41	39	21
14	28	32	32	14	14	28	22	28	35	26	24	34	20	20
15	54	46	54	50	54	44	24	26	31	22	27	43	25	33
16	30	41	35	26	22	31	24	25	75	54	59	59	44	51
17	10	15	17	17	19	17	11	11	28	6	9	28	18	16
18	42	51	52	44	45	28	31	32	68	57	78	42	61	58
$\bar{X}$	21.7	23.5	24	22.9	20	19.2	16.8	18.9	37.5	27.8	29.4	26.2	25.7	26.2
SEM	3.7	4	3.8	3.3	3.6	3	2.6	3.3	4.9	5	5.1	4	3.7	4.4
<u>log transformation of raw data</u>														
X	1.215	1.24	1.265	1.27	1.195	1.18	1.12	1.15	1.50	1.33	1.35	1.32	1.34	1.34
SEM	0.09	0.09	0.09	0.07	0.08	0.08	0.08	0.09	0.07	0.08	0.09	0.1	0.06	0.06

Table 419

Plasma motilin (pmol/l) in 18 obese women at 36 weeks of pregnancy. The glucose was given at time 0 and the meal at time 180

Ser	-30	0	30	60	90	120	150	180	218	240	270	300	330	360
1	86	82	77	72	72	80	88	88	124	134	86	96	102	100
2	18	18	9	10	7	10	9	12	20	19	12	20		
3	50	52	68	55	30	35	21	13	38	34	57	35	22	62
4	131	135	93	80	58	46	50	60	84	69	40	38	59	76
5	44	36	23	14	25	16	26	25	41	24	24	20	41	28
6	21	24	26	18	11	29	20	27	68	78	47	68	63	57
7	100	122	97	84	92	87	94	82	165	123	125	136	124	124
8	54	59	50	54	40	44	15	16	44	45	48	27	26	22
9	58	48	30	16	12	6	7	8	36	43	20	16	40	35
10	29	25	34	35	29	12	20	21	35	22	26	36	22	46
11	58	51	65	30	25	27	43	42	70	66	57	40	48	56
12	70	63	41	21	16	26	35	36	57	102	73	60	60	63
13	113	110	94	106	106	53	46	44	89	72	60	78	60	55
14	80	82	51	60	55	60	59	59	70	43	48	57	71	48
15	72	78	53	53	60	72	81	80	89	78	84	53	83	53
16	97	98	92	78	84	60	42	60	120	124				
17	38	40	28	38	39	39	26	21	27	32	32	46	30	40
18	98	84	86	80	87	52	55	52	114	85	114	92	115	73
X	67.6	67.1	56.5	50.2	47.7	41.9	40.9	41.4	71.7	66.3	56	54	60.4	58.6
SEM	7.8	8.3	6.9	7	7.6	5.8	6.4	6.2	9.6	8.9	8	8	8.3	6.7
log transformation of raw data														
X	1.77	1.765	1.68	1.611	1.56	1.53	1.51	1.52	1.79	1.75	1.675	1.66	1.72	1.73
SEM	0.06	0.06	0.07	0.08	0.08	0.08	0.08	0.08	0.06	0.06	0.07	0.07	0.06	0.05

Table 420

Plasma motilin (pmol/l) in 18 obese women at 1 week post-partum. The glucose was given at time 0 and the meal at time 180.

In preterm infants plasma motilin rose from the cord level of 13 ± 4 pmol/l to basal concentrations of 89 ± 16 pmol/l on day 6 ($p < 0.01$) and to 150 ± 30 pmol/l on day 24 ($p < 0.001$), (Figure 419). In contrast, by day 6 preterm infants who had not been fed orally did not show this pronounced motilin increase; their mean plasma motilin level was 24 ± 4 pmol, significantly lower than the orally-fed preterm infants at this time after birth ($p < 0.001$), (Figure 420).

Breast-fed term infants aged 6 days had motilin levels (26 ± 3 pmol) similar to the cord level, but by day 16 the level had risen to 123 ± 24 pmol, $p < 0.001$ (Fig 419). Basal motilin was higher in 6-day-old preterm infants than in term ones ($p < 0.001$).

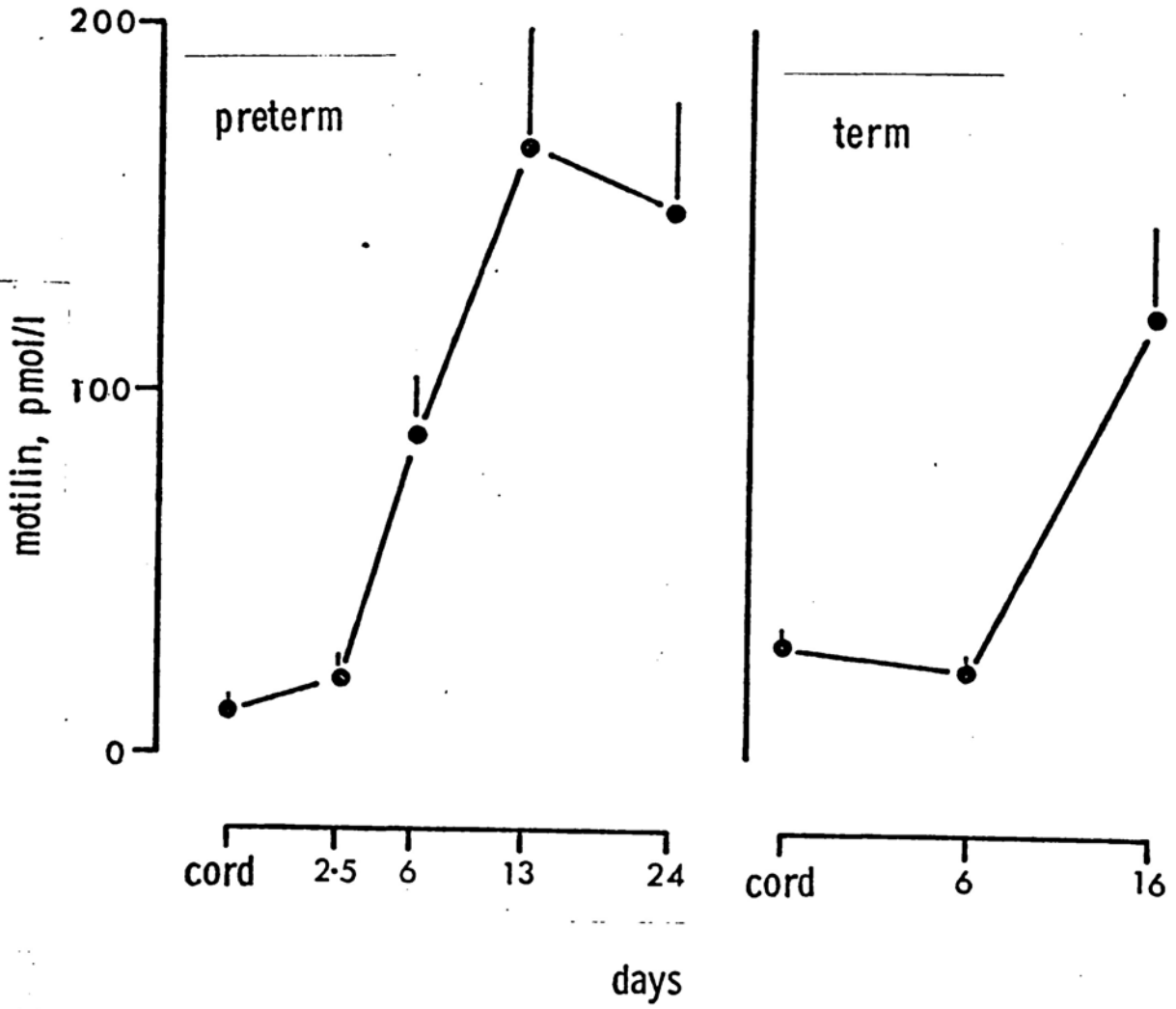


Figure 419

Plasma motilin levels in preterm and term babies.

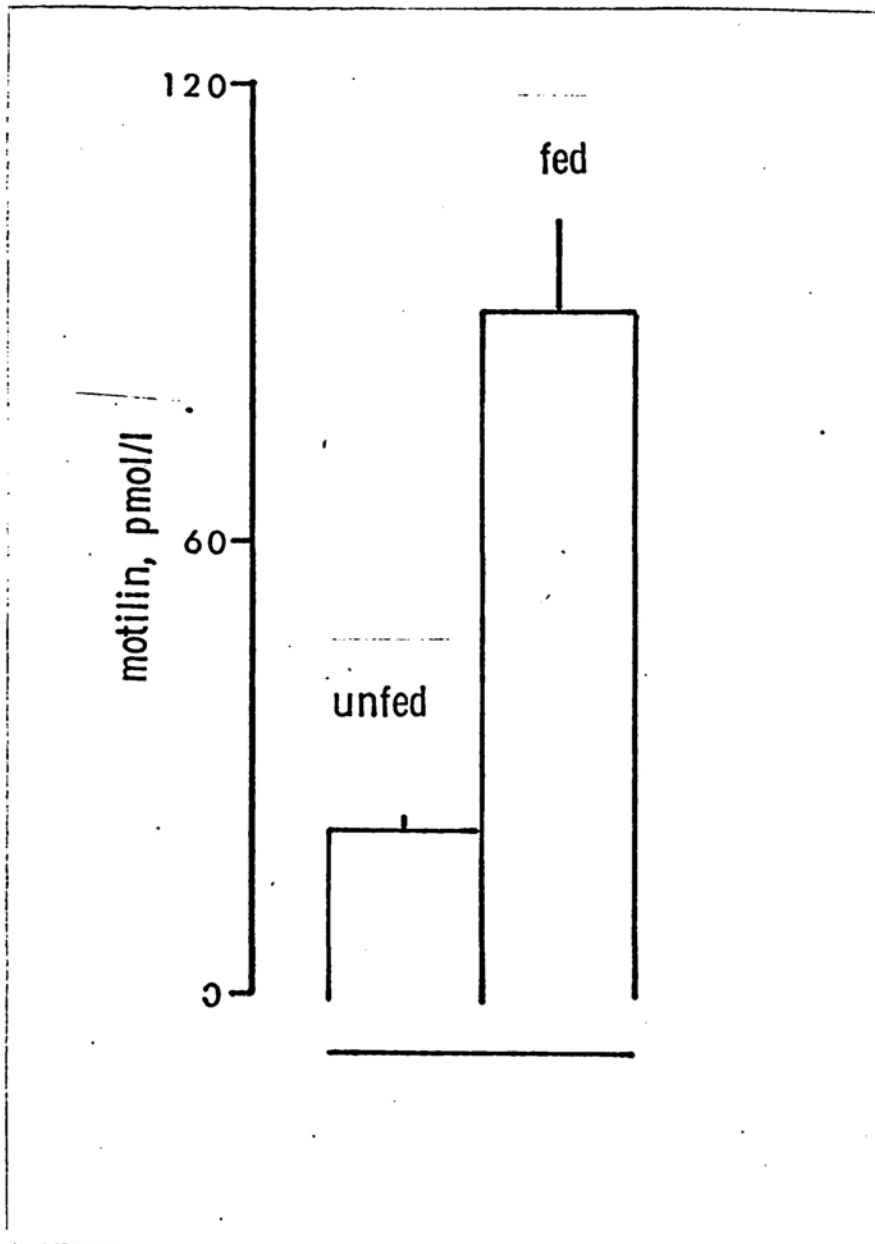


Figure 420

Plasma motilin levels in 6-day-old preterm babies, either orally fed (fed) or those fed IV (unfed).

Molecular forms in plasma

Recovery of total motilin immunoreactivity during chromatography was always between 80 and 100%.

Figure 421 shows the gel chromatographic profiles of plasma motilin-like immunoreactivity following the various stimuli. In the basal state two immunoreactive motilin peaks were apparent. Peak I eluted at a position ($K_{av}=0.16$) suggesting a large molecular size substance whereas peak II coeluted with the porcine motilin standard ($K_{av}=0.67$). After ingestion of the test breakfast the concentration of both immunoreactive motilin peaks rose significantly (peak I 29 ± 7 fmol/ml to 50 ± 15 fmol/ml, $p < 0.05$, and peak II 33 ± 5 fmol/ml to 71 ± 11 fmol/ml, $p < 0.005$). Following the water load peak I rose significantly from 20 ± 3.5 fmol/ml to 31 ± 3.5 fmol/ml, $p < 0.025$ and peak II from 21 ± 3.5 fmol/ml to 51 ± 9 fmol/ml, $p < 0.01$. Both peaks also rose significantly, following ingestion of the cream (peak I 13 ± 1.5 fmol/ml to 34 ± 3 fmol/ml, $p < 0.02$, and peak II 14.5 ± 2 fmol/ml to 58 ± 5 fmol/ml, $p < 0.025$). In contrast, after glucose both motilin peaks were significantly suppressed (peak I 51 ± 6 fmol/ml to 18 ± 4 fmol/ml $p < 0.001$ and peak II 41 ± 7 fmol/ml to 9 ± 2 fmol/ml, $p < 0.025$). Infusion of somatostatin resulted in virtual disappearance of both motilin peaks. Following infusion of porcine motilin, peak II immunoreactivity rose as expected (50 ± 9 fmol/ml to 117 ± 18 fmol/ml, $p < 0.001$) but peak I was significantly suppressed (51 ± 5 fmol/ml to 30 ± 4.5 fmol/ml, $p < 0.005$).

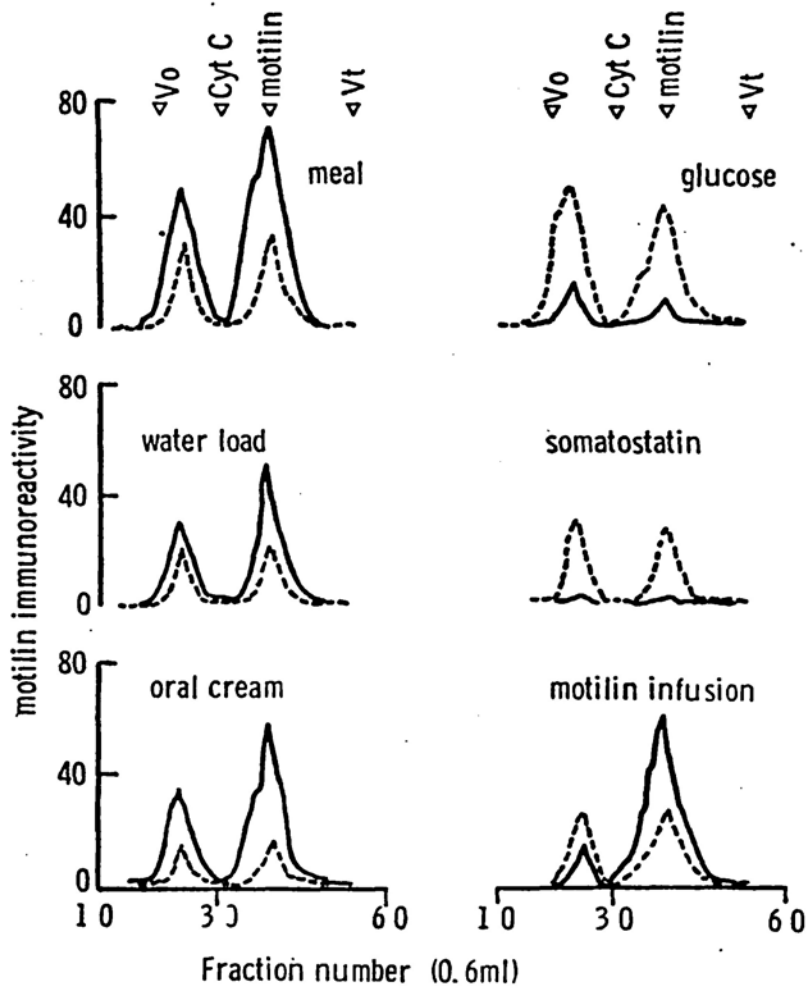


Figure 421

Elution profiles of plasma immunoreactive motilin following various stimuli: Test meal (top left), water load (mid left), cream (bottom left), Glucose (top right), somatostatin (mid right) and porcine motilin (bottom right). Broken lines depict fasting profiles and continuous lines, profiles after stimulation.

Table 421 shows the motilin immunoreactivity (fmol/ml) in 26 plasmas analysed chromatographically using antibodies M1 and M25. With antibody M1 the mean concentration of these plasmas was 210 ± 27.7 fmol/ml and with antibody M25 was 87.3 ± 6.4 fmol/ml. After chromatography two immunoreactive peaks were detected with M1. Antibody M25 only measured one of these peaks (peak II, $K_{av}=0.67$, see Figure 422). The mean immunoreactive motilin concentration eluting at a K_{av} of 0.16 (peak I) was 112 ± 16.4 fmol/ml and at a K_{av} of 0.67 (peak II) was 90.2 ± 15.8 fmol/ml. Figure 423 shows the relationship between the motilin immunoreactivity, as measured by M25, and peak II motilin (following chromatographic analysis and measured by antibody M1). A highly significant correlation was observed ($r=0.956$). Figure 424 shows the relationship of the difference (M1-M25) with peak I motilin immunoreactivity (as measured with M1 following chromatographic analysis). Again, a significant correlation was observed ($r=0.951$). These figures were plotted logarithmically as both peak I and peak II values follow a skewed distribution. Figures 425 and 426 show the distributions of peak I and peak II when plotted in absolute values (pmol/l) and following 'normalisation' (converting values to logarithms).

	<u>M1</u>	<u>M25</u>	<u>M1-M25</u>	<u>Peak I</u>	<u>Peak II</u>
1	122	37	85	63	56
2	102	30	72	51	46
3	560	282	278	268	291
4	405	137	268	210	178
5	200	95	105	72	120
6	185	60	125	105	70
7	205	115	90	129	74
8	225	75	150	159	61
9	190	125	65	65	126
10	70	35	35	40	30
11	70	40	30	20	50
12	255	127	128	84	145
13	125	15	110	76	17
14	220	80	140	162	46
15	290	160	130	122	162
16	580	360	220	226	331
17	167	85	82	100	67
18	130	97	33	25	99
19	205	95	110	109	96
20	265	85	180	170	98
21	85	20	65	42	21
22	95	45	50	29	44
23	70	15	55	25	28
24	370	27	243	329	41
25	70	22	48	38	38
26	205	5	200	195	10
X	210	87.3	123	112	90.2
SEM	27.7	16.4	16.4	16.4	15.8

Table 421

Motilin immunoreactivity, in 26 plasmas, using antibodies M1 and M25. Columns. 'Peak I' and 'Peak II' represent the motilin values achieved following chromatography. All values are expressed as fmol/ml.

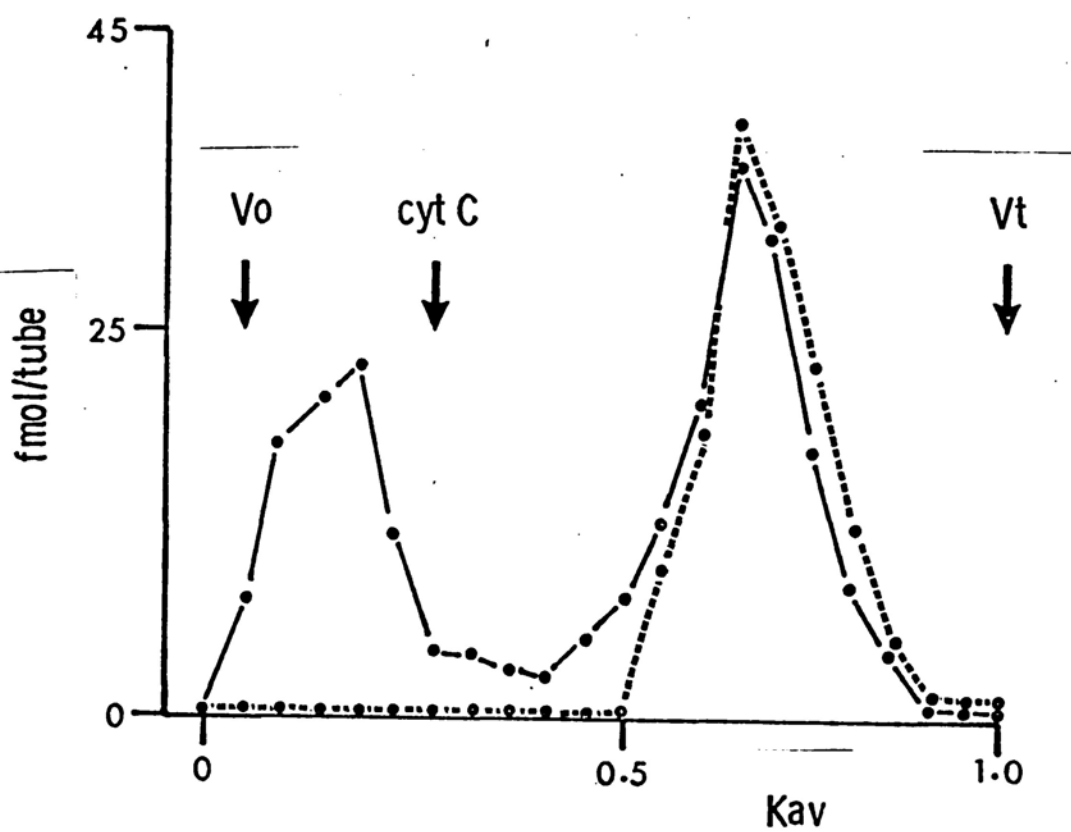


Figure 422

Elution profile of plasma motilin using antibody M1 (solid line) and M25 (broken line)

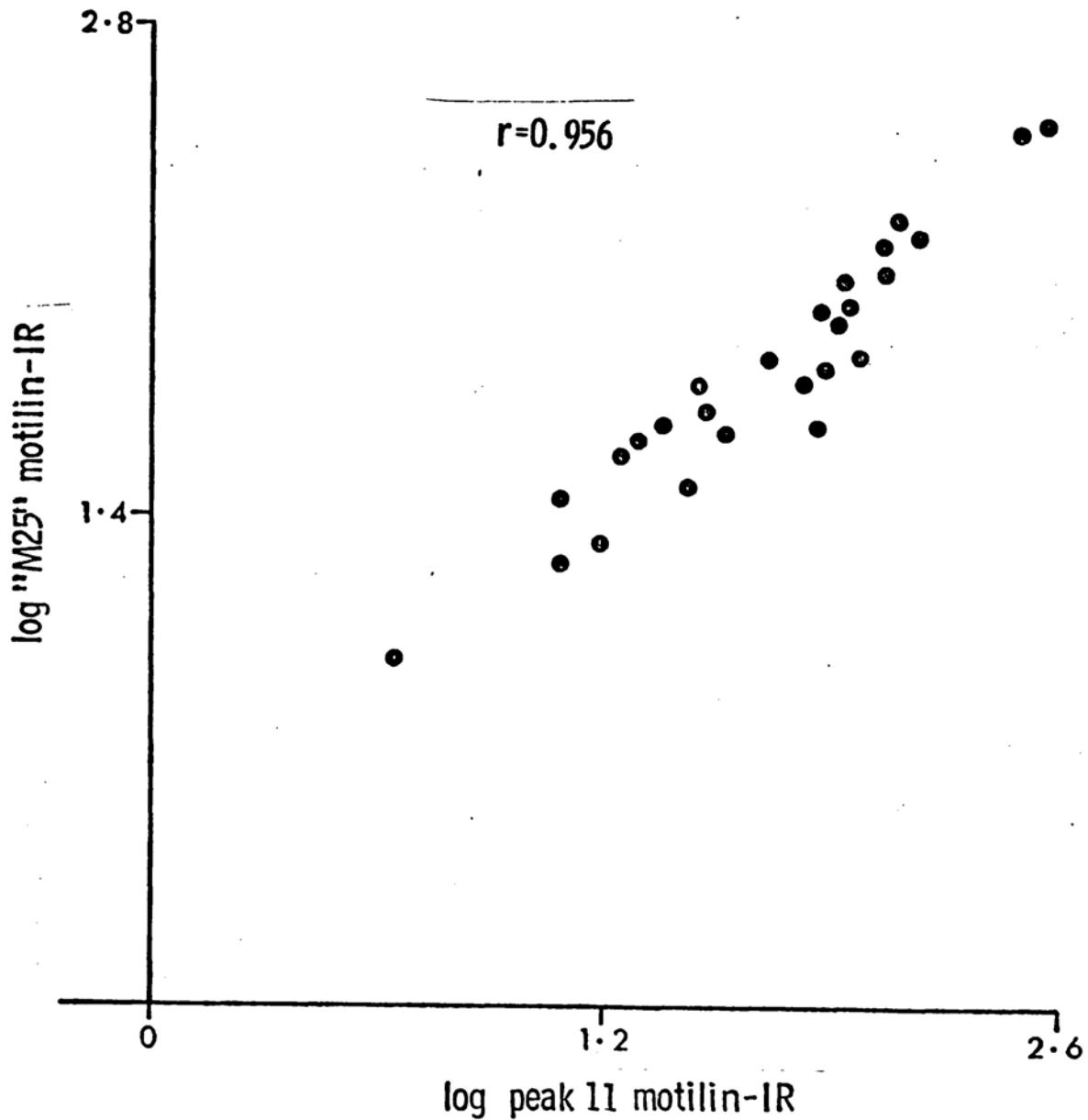


Figure 423

Correlation between motilin immunoreactivity (measured with M25, x-axis) and peak II motilin (measured with M1, y-axis).

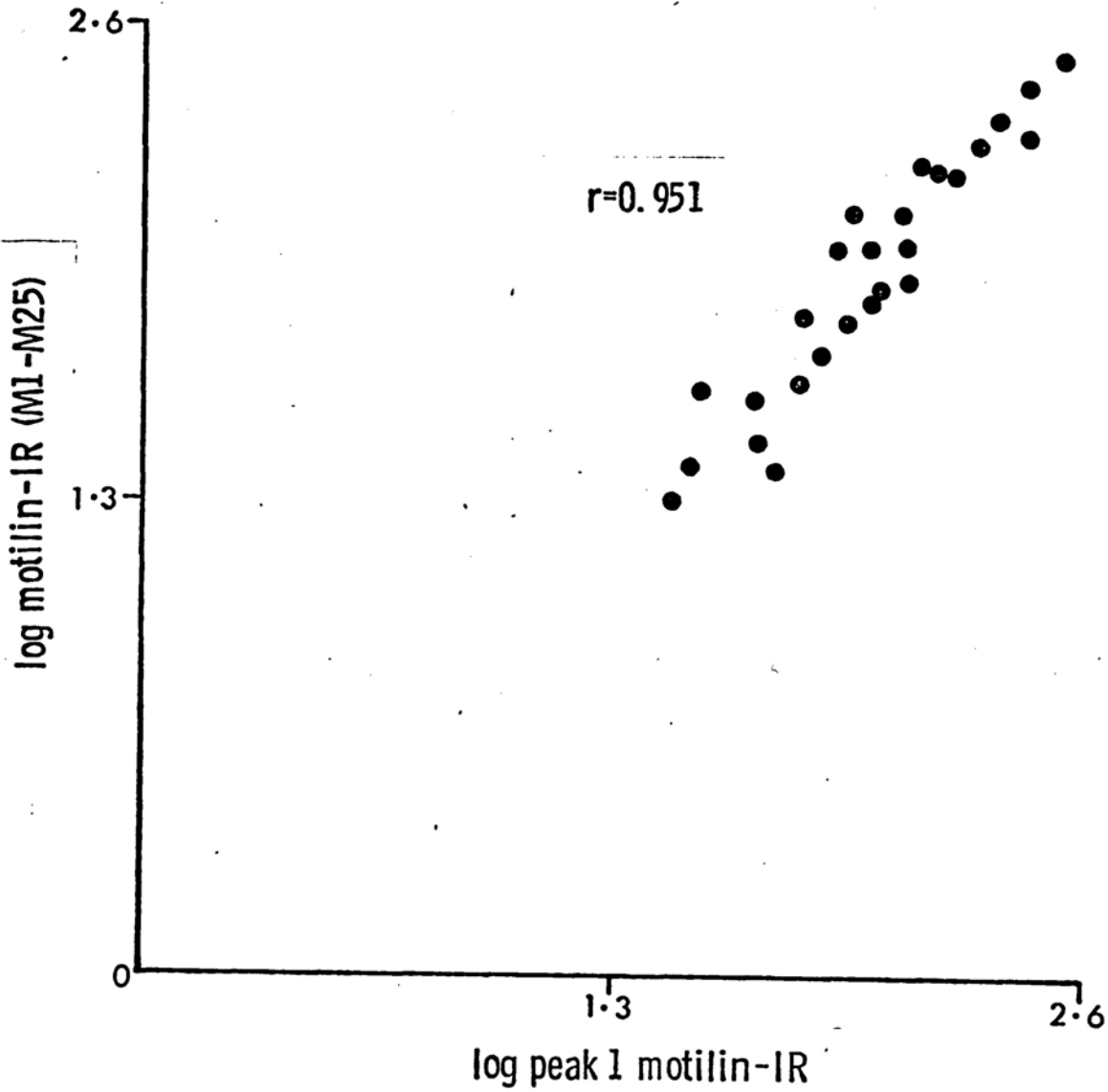


Figure 424

Correlation between peak I motilin (measured by M1, y-axis) and the difference between the motilin immunoreactivity (M1-M25, x-axis).

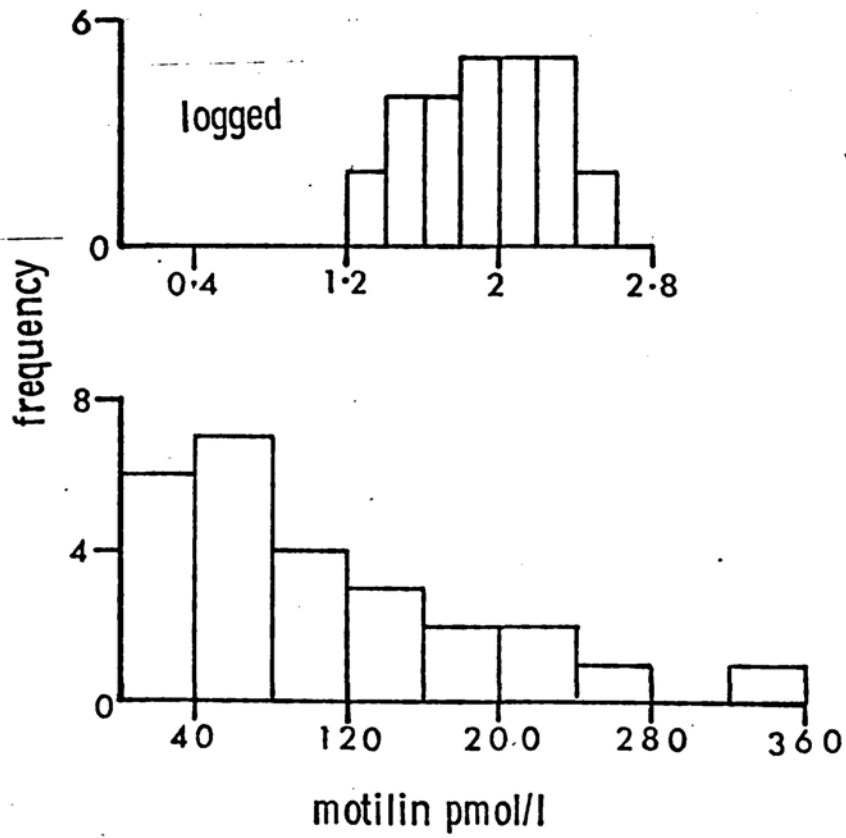


Figure 425

Log transformation (top) of peak I motilin values.

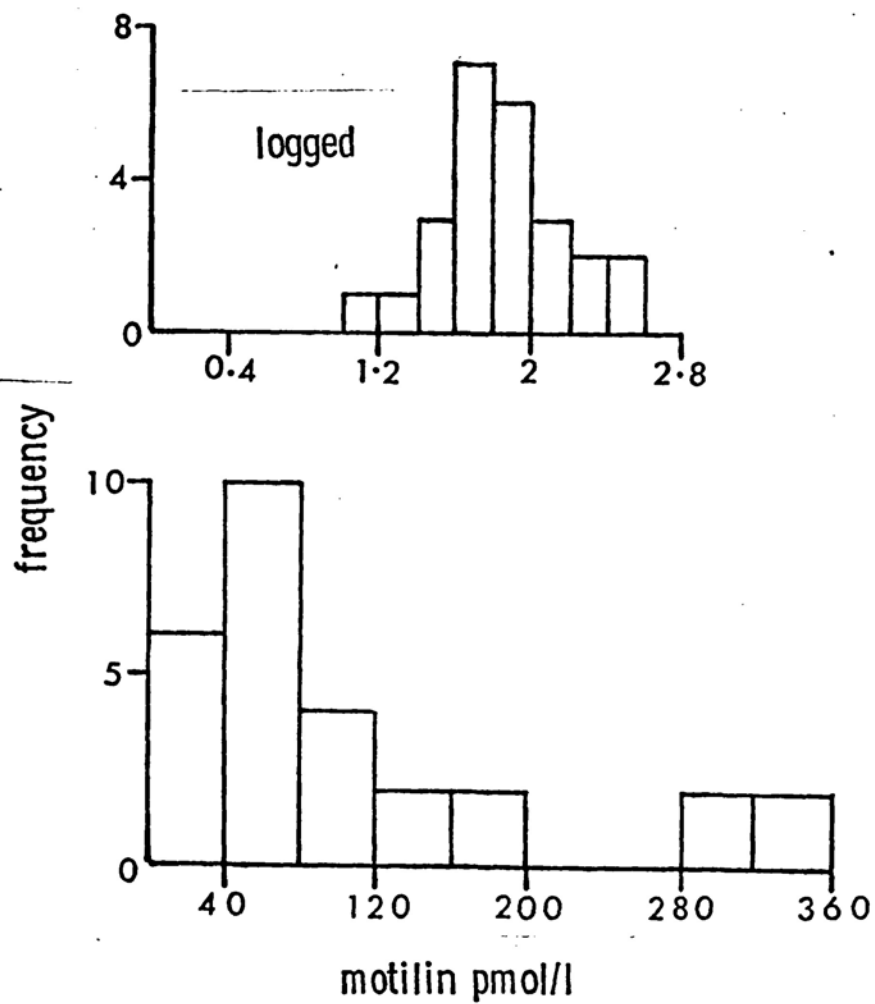


Figure 426

Log transformation (top) of peak II motilin values.

DISCUSSION

Release of motilin

The investigation of the release of any hormone in the circulation is of prime importance in the understanding of its physiology. It was therefore essential to investigate the mechanisms controlling the release of motilin and also to determine the magnitude of this release (necessary information for exogenous infusion studies - see Chapter 5). It has been proposed by Grossman (73), that for any gut peptide to achieve hormonal status its release into the circulation following ingestion of a meal must be demonstrated. The data presented here show that motilin is indeed released postprandially. This release, however, is small and transient, lasting for about 30 min. The magnitude of the release (25pmol/l from a basal of 48pmol/l) compares unfavourably with the release of other hormones, such as insulin, pancreatic polypeptide and gastrin. The mechanisms involved appear to be complex and not well understood. Different food components appear to either stimulate (fat) or inhibit its release (glucose). Thus meals differing in nutriment composition may give contradictory results. Recent reports by Yanaihara (99) and others (89, 84) confirm these results. These authors showed that motilin release was stimulated following ingestion of fat but was inhibited by glucose. Collins and colleagues (90) reported that motilin release was significantly stimulated by oral fat but they found no change with a mixed meal. This meal however contained only 8g fat as compared to 22g used in the present studies.

When glucose was given intravenously, motilin was suppressed to an equivalent degree to an oral load while intravenous lipids produced a rise in motilin. This has led to the speculation (91) that the circulating concentration of intravenous nutrients can, in some way, act on the motilin cell to control release and thereby influence gastrointestinal motor activity and regulate the absorption of further nutrients. Infusion of a mixture of

aminoacids resulted in a suppression of plasma motilin, although ingestion of protein elicited a small rise (not statistically significant). This observation led us to investigate the effect of gastric distension on the release of motilin. In the first series of experiment large volumes of water were ingested and these caused the highest, to date, elevation of motilin (60 pmol/l). This rise did not appear to be under vagal control since atropine did not blunt the response (the peak rise however appeared to be delayed). This series of experiments suggested that gastric distension may, somehow, cause the release of motilin. However, a number of variables were also introduced, such as osmolarity, pH and duodenal distension. As motilin is present in very low concentrations in the stomach, the highest concentrations being in the duodenum, we also investigated the effect of duodenal distension (a pilot study in two subjects) by means of a rubber balloon air-filled at a pressure of 30 cm³. This however, did not result in any motilin change. The use of air-filled balloons positioned in the antrum confirmed that antral distension does, indeed, cause release of motilin. This rise did not appear to be under adrenergic influence, as propranolol and metoprolol (β_1 - β_2 and β_1 adrenergic blockers) did not significantly influence this release. Recent evidence suggests that apart from the two classical nervous systems (cholinergic and adrenergic) there may be a third component, the peptidergic system (42). Putative neuromodulators include bombesin, vasoactive intestinal peptide, somatostatin and many others. As bombesin is found in high concentrations in the stomach (43, 94) and may have been a mediator of the release of motilin following antral distension, we investigated its effect on motilin by an intravenous infusion. It was thus shown that bombesin elicited a significant motilin release supporting the above hypothesis. Other, as yet untested, factors may have also contributed to the release of motilin following distension. The finding that other polypeptide

hormones, released by a meal, influence the release of motilin complicates the picture even further. Both somatostatin (95) and pancreatic polypeptide (96, 97) have been reported to be released by fat, a potent stimulus of motilin release. Yet infusion of either of these peptides result in gross inhibition of motilin (over 80%). Insulin also appears to significantly suppress motilin. It is thus apparent that the release of motilin is the net result of many agonists and antagonists acting together (such as food composition, neural influences and other polypeptide hormones). The finding that basal motilin is under vagal (atropine sensitive) control awaits further investigation.

Molecular forms

Motilin, like many other gut hormones (98), appears to exist in more than one molecular form in the circulation. Chromatographic analysis of plasmas revealed the presence of two molecular forms, corresponding in their elution position with those present in the intestine (peak I, $K_{av}=0.16$, peak II, $K_{av}=0.67$). Both these peaks changed in parallel following various stimuli. They both rose following ingestion of a meal, fat and water and were suppressed by glucose and somatostatin. However, infusion of porcine motilin resulted in the expected rise in peak II, but a significant inhibition of peak I motilin, perhaps suggesting the possibility of a negative feedback control mechanism. This is an aspect that has been very poorly studied in regards to other heterogeneous hormones. The only example in the literature is the suppression of plasma C-peptide by exogenous infusion of insulin (99, 100). Whether the two motilin peaks are related in the same way as the insulin forms must await the future purification of the larger form. No other investigations on plasma motilin heterogeneity have been published.

The finding of a highly significant correlation between peak I (as measured with MI following chromatographic analysis) and the motilin difference (MI-M25) suggests that it would be possible to investigate peak I release in both health and disease, using these two antibodies (a situation that is similar to enteroglucagon assay (101), which employs a totally reacting antibody and a highly pancreatic glucagon specific antibody, the difference between values measured by the two antibodies being enteroglucagon).

Motilin in pregnancy

There is a general relaxation of smooth muscles during pregnancy (102). It is believed, for example, that reduction of stomach muscle tone and lower oesophageal pressure can result in delayed gastric emptying (103) and regurgitation of gastric acid (104), which can give rise to recurrent heartburn and epigastric discomfort. Reduced smooth muscle tone also occurs in the large bowel (102), and is thought to explain the constipation which is often seen in pregnancy. In addition there is delayed emptying of the gall-bladder (105,106). Because of the stimulating effects of motilin on gastrointestinal smooth muscle (107), we investigated the possibility of hypomotilinaemia during pregnancy. The results showed that plasma motilin levels were significantly lower at 16 and 36 weeks of pregnancy when compared to those at 1 week postpartum. It is thus possible that some of the symptoms, seen during pregnancy, may be due to the suppression of motilin.

Motilin in early life.

High basal plasma motilin levels in children have been previously reported by Mitznegg et al (108). The results presented here show that, in preterm infants, this elevation is a result of postnatal events, and may be related to enteral feeding, since those preterm infants who were maintained on intravenous dextrose for 6 days had no appreciable rise in motilin. This observation, however may be complicated by the presence of hyaline membrane disease.

The postprandial falls in motilin are similar to the late falls after a meal in the adult; in adults, however, there is a small initial rise, but this could have been missed in the neonates studied by not taking immediate postprandial samples. The high motilin levels before feeds could be related to the so-called 'hunger contractions' (109,110), of the stomach that have been recorded mechanically in term and preterm neonates in the interprandial phase. It was found that breast-fed, 6-day-old, term infants and preterm infants at 2.5 days, did not show any motilin response to feeding. It is possible that such responsiveness requires threshold 'priming' of the gut with food. This could explain why breast-fed infants differ from formula-fed infants, who show a postprandial fall from higher basal levels, since the latter have a larger milk intake during the first 6 days of life (though feed volumes in the two groups were similar during the study itself). Another interesting possibility, however, is that the differences between breast and bottle-fed infants were due to differences in feed composition.

CHAPTER 5

PHYSIOLOGY OF MOTILIN

Introduction

The classical methods of investigating endocrine physiology, such as extirpation of the gland (resulting in a deficiency syndrome) or injecting extracts (resulting in excess symptoms) are not applicable to the gut. The gut is a diffuse endocrine organ, where the different hormone producing cells overlap in distribution and are intermingled with non-endocrine cells. Thus a circumscribed area of unihormonal tissue is not available and endocrine ablation or injection of extracts is, thus, impossible. One mode of investigation available is to infuse, into fasting volunteers an amount of hormone that mimics levels seen after some physiological stimuli and then to assess any effects in the light of known pharmacology. Although this does give useful information it is by no means conclusive. The control of biological functions is usually a delicate balance between numerous agonists, antagonists and modulators (for example, 20 different factors are known to affect gastric acid production). Thus the isolated introduction of a single hormone may not be wholly physiological. In order to verify the importance of one regulating substance in a complex control system, it is necessary to investigate the effect of a very small change in its concentration in the correct functional setting. This however, is beyond current methodology. Other drawbacks in these investigations, are that very often the hormone that is infused has been purified from a different species from the recipient. For example, a porcine hormone infused in a pig may have a different effect when it is infused in man. Also, the majority of gut hormones exist in many molecular forms of perhaps different potencies and unknown actions. Thus results gained from infusions of a single molecular form may not be representative of the true physiological role of the hormone studied.

Very often, information of the physiological role of a hormone can be obtained by studying human disease. Thus tumours of gastrointestinal origin producing vast amounts of a single hormone can yield useful insight into its physiology.

Studies on the pharmacological actions of motilin started soon after it was successfully synthesised by Wunsch et al in 1973 (17). In vitro experiments demonstrated that motilin had contraction promoting effects on the smooth muscles of the gastric antrum, duodenum and colon (26,27,28). These stimulatory effects were not antagonised by anticholinergic drugs, antihistaminic agents or ganglion blocking agents and it was therefore proposed that motilin directly stimulates the smooth muscle (28). This view was supported by the fact that its effect was inhibited only by verapamil, a compound thought to inhibit the influx of calcium and thereby reduce the activation of myofibril-ATPase.

Subsequent in vivo studies in dogs indicated that motilin infusions induced the formation of the interdigestive myoelectric complex (34,35) the so called "housekeeping" intermittent waves of contraction, that sweep down the gut and are presumed to act to ensure removal of unwanted secretions and debris. Motilin was also shown to produce phasic pressure changes in the lower oesophageal sphincter, both in man (44) and dog (41-43). Ruppin and colleagues (37) studied the effect of motilin on intestinal transit time and found that infusion of 2.5pmol/kg/min motilin caused a 50% decrease of the transit time. These same investigators also studied the effect of motilin on gastric emptying. They reported (38) that motilin greatly delayed the gastric emptying of a test solution consisting of 10% mannitol in 0.5M citric acid-phosphate buffer pH3.0. Plasma motilin levels achieved during all the above infusion experiments were not reported.

The aim of this chapter is to describe the studies undertaken in order to investigate the role of motilin, with constant monitoring of plasma levels.

1. Gastric emptying of a mixed meal.

Subjects

Five healthy male subjects (aged 25-35 years) were studied after an overnight fast. The rate of gastric emptying of a 'solid meal' marker, mixed with the test breakfast was measured on four separate days, in each subject. On two days, a concomitant intravenous infusion of normal saline (0.15M saline with 200 μ mol/l human serum albumin) was given whilst on the other two days motilin was infused. Care was taken to ensure that the subjects were unaware which infusion was being given either before or after the study. The research protocol was approved by the Royal Postgraduate Medical School's Ethical Committee and informed written consent was obtained from all subjects.

Gastric emptying method. The gastric emptying method used was as outlined by Cowley et al (111). After an overnight fast the subjects were given cornflakes, 300 ml of milk, tea, two scrambled eggs and two slices of buttered bread (65g carbohydrate, 26g fat and 29g protein, total volume 500 ml). The subjects ate the breakfast in less than 5 mins. Caesium -129 was absorbed onto a zirconium phosphate resin (B10-RAD ZP-1, 100-200 mesh) to provide a solid inert carrier and 100 μ Ci was given with the cereal. With the subjects already positioned under a γ -camera (Nuclear enterprises Mark IV with a high energy parallel hole collimator, connected to a Hewlett-Packard 2100A computer), in a sitting position, collection of the data started immediately following complete ingestion of meal. Recording was continued for 90 mins. Data were stored in digital form on a magnetic tape and the rate of decrease of counts (after decay correction, $t_{1/2}$ 32 hours for Cs 129) in the stomach area was computed against time. The selection of the stomach area was based on the distribution of activity in the first four minutes of acquisition of data, but the position of the stomach was checked throughout to confirm that it remained wholly within the area of count analysis. The assessment of the gastric area was performed by an independent observer.

Motilin infusion. Pure natural porcine motilin was obtained from Professor JC Brown of the Department of Physiology, University of British Columbia. After a basal period of 20 mins, motilin was infused at a dose of 0.34 pmol/Kg/min for one hour and the dose was then increased to 0.68pmol/Kg/min and maintained for a further hour until the completion of the experiment. The motilin was dissolved in 0.15M saline containing 200 µmol/l human serum albumin and was delivered using a syringe ram pump, connected to a "butterfly" 19G needle, which was inserted in an antecubital vein (left arm). The test breakfast was given 30 mins after the start of the low infusion dose. Throughout the experiment, 10 ml aliquots of blood were withdrawn at 10 min intervals, from an antecubital vein catheter (right arm) into chilled heparinised tubes containing 4000 KIU of aprotinin, then rapidly centrifuged and the plasmas frozen. On completion of the experiment the infusion of motilin was discontinued and blood samples were collected every minute for 7 mins in order to determine the initial half life of motilin decay.

Hormone radioimmunoassay. Plasma hormone concentrations were estimated using radioimmunoassays to gastrin (112), pancreatic polypeptide (PP) (113), gastric inhibitory peptide (GIP) (114), glucagon (115,116), VIP (117), insulin (118) and motilin (using antibody M1). Blood glucose was measured using the alkaline-ferricyanide method (119) as adapted for the autoanalyser. Antisera to gastrin were raised in rabbits using synthetic human gastrin I conjugated by carbodiimide to BSA as the hapten. Iodination of gastrin I was carried out using chloramine T oxidation. The label was purified on an ion exchange resin (QAE-A25) using a gradient elution technique. The specific activity of the label was 76 Bq/fmol. The antibody crossreacted equally with G17 and G34 and was used at a final dilution of 1/160,000. The pancreatic polypeptide (PP) antibody was raised using human PP as the immunogen. The label was prepared using lactoperoxidase oxidation and was purified on G50 sephadex.

Calculations. Hormone concentrations and emptying rates are expressed as mean $\pm$ standard error of the mean. Paired t-test (following log transformation) was used for statistical determination.

The pharmacokinetics of motilin were calculated using the incremental plateau principle described by Goldstein et al (120). To determine the half life of decay ($t_{1/2}$) of motilin each subjects' mean basal level was subtracted from all subsequent values. The post infusion values were expressed as a percentage of the steady state final plateau incremental level and then converted to their natural logarithms. These values were plotted against time. The metabolic clearance rate (MCR) was calculated by dividing the infusion rate (measured by radioimmunoassay, in pmol/Kg/min) by the steady state increment of plasma motilin (pmol/ml). The apparent space of distribution (DS) was calculated from the formula : $DS(ml/Kg) = MCR \times t_{1/2} \times 1.44$.

2. Gastric emptying of glucose and fat

Subjects

Seven healthy subjects, 6 male and 1 female (aged 22-35 years) of normal weight (75 ± 5 kg) were studied. The rate of gastric emptying of a radioisotope marker, technetium 99^M - tin colloid (100 μ Ci) mixed with 50g of 25% glucose was measured on 2 separate days. On one day a concomitant intravenous infusion of normal saline (with 200 μ mol/l HSA) was given while on the other day motilin was infused.

A further group of 5 subjects, all male (aged 26-37 years) of normal weight (73 ± 4 Kg) were given technetium 99^M - tin colloid (100 μ Ci) mixed with 50g of double cream (25g fat), mixed in 200 ml of water. On one day an intravenous infusion of normal saline (with 200 μ mol/l HSA) was given while on the other day motilin was added. All infusions were given blind and in random order.

In vitro validation. Gastric juice was collected from subjects undergoing routine pentagastrin tests. Two hundred millilitres of the juice were then mixed with 200 ml of diluted double (24g fat) to which 100 μ Ci of technetium 99M tin colloid was already added. The mixture was incubated for 30 min at 37°C; it was then centrifuged at 2000 rpm for 30 min (Coolspin, MSE, England). The resultant aqueous and fat layers were then counted in a gamma-counter (Nuclear Enterprises, England) for their radioactive content.

Gastric emptying method. The gastric emptying method was the same as described on page 161. Ingestion of either of the test meals (glucose or double cream) was completed within 2 min. With the subjects already positioned under the gamma camera in sitting position collection of the data started immediately following complete ingestion of the meal. The gastric emptying was monitored for 60 min. Data were stored on a magnetic tape and the rate of decrease of counts in the stomach was computed against time after correction for radioactive decay ($t_{1/2}$ for technetium is 6 hours).

Motilin infusion. Following a basal of 20 min, natural porcine motilin was infused at a dose of 0.2 μ mol/Kg/min for 80 min. The test breakfast was given 20 min following the start of the infusion. Throughout the experiment 10ml aliquots of blood were withdrawn at 5 min intervals, centrifuged and the plasmas frozen until assay. Plasma insulin concentrations were estimated by RIA (118) using an antibody to bovine Insulin (Wellcome) and bovine Insulin I125 (Wellcome). Plasma glucose was measured using the glucose oxidase method (121).

Plasma chromatography. Plasma aliquots (1 ml taken basally and 30 min after ingestion of the cream in all five subjects both during placebo and motilin infusion) were applied to the top of a 0.9x60cm column containing Sephadex gel, G50 superfine. The elution buffer used was 0.01M EDTA 0.06M phosphate pH7.4

containing 1% human serum albumin. Columns were run at 6ml per hour at 4°C and were precalibrated with dextran blue, cytochrome C, sodium ^{125}I and porcine motilin. Dextran blue and cytochrome C were included with each sample run as internal markers. Fractions were collected every six minutes and each assayed for motilin immunoreactivity.

3. Motilin and the interdigestive migrating motor complex in man

Method

Intraluminal pressures were measured (86) by means of three polyethylene catheters (PE 205, Clay Adams, div of Becton, Dickinson and Co., Parsippany, N.J.) with side openings 25cm apart. The catheters were perfused continuously at a rate of 0.19ml/min (Harvard pump, Harvard apparatus Co., Inc., Millis Mass). External pressure transducers (EMT 34, Elema Schönander, Stockholm, Sweden) were used and the tracings were recorded on a polygraph (Mingograph 81, Elema Schönander). The catheter assembly included two other tiny polyethylene tubes (PE 60, Clay Adams) which ended in a mercury-filled latex bag 15cm below the most aboral recording orifice. The mercury-filled bag facilitated the passage of the catheter system through the small intestine whereas the two additional catheters made it possible to aspirate the mercury. The proximal orifice was positioned under fluoroscopic control in the distal part of the duodenum about 10cm above the angle of Treitz.

Analysis of pressure records

The first step of the analysis consisted of counting the number of pressure waves per minute throughout the recording period (86). Only waves of 5mmHg or more were taken to account. In a second stage the interdigestive motor complexes were identified using the following criteria for identification of an activity front :-

- a. appearance of an uninterrupted burst of pressure waves which were produced at a rhythm of about 12 per min at the level of the angle of Treitz;

- b. aboral progression of the activity front, as indicated by its sequential passage over all three, or over the distal two recording orifices;
- c. a period of complete quiescence after the activity front.

The activity fronts were identified independently by two observers. The final step of analysis consisted of determining the duration of the bursts of rhythmic contractions at different levels (duration of activity fronts), the frequency of the contraction waves during the periods of rhythmic activity, the progression velocity of the activity front, the calculated length of the activity front (duration x progression velocity), and the duration of the complete cycle of the motor complex. As this phase of analysis is subject to observer bias and requires the drawing of marks on the tracings, it was carried out jointly by three subjects. The data were analysed statistically by means of Student's t-test.

Subjects. The relation between plasma motilin levels and intestinal motor activity was investigated in twenty three subjects, (studied at the department of Medicine, University of Erlangen, Germany).

Infusion of motilin. Pure natural porcine motilin (Kindly supplied by Professor J.C. Brown, Vancouver, Canada) was used for intravenous infusion. Informed consent to carry out motilin infusion studies was obtained from 16 normal volunteers aged 18 to 26 years. Prior to the administration of motilin, blood was drawn every 10 min until an activity front was observed. A saline infusion was given from the beginning of the experiment. The infusion of motilin at nominal rates of 0.4, 0.8, 1.6, 3.2 and 6.4 pmol/kg/min was started one or two min after the activity front appeared at the level of the distal recording orifice. Blood was drawn every 5 min during the 30 min infusion period, and every 10 min for an additional 30-60 min thereafter. Statistical analysis of the data was performed by student's t-test for paired data (after 'log transformation').

RESULTS

Gastric emptying of the mixed meal

Motilin levels. Figure 501 shows the plasma motilin levels achieved during the saline and motilin infusions. The mean zero time motilin value during the motilin day was not significantly different from the corresponding value during the saline day (68 ± 13 pmol/l and 61 ± 6 pmol/l, respectively). Infusion of saline did not result in a significant alteration of plasma motilin levels. Following infusion of motilin (0.34 pmol/Kg/min) plasma motilin rose significantly ($p < 0.01$) to 110 ± 11 pmol/l at 30 min. This value was significantly higher ($p < 0.005$) than the corresponding value during the saline infusion. Ingestion of the meal, during the control saline infusion, resulted in a small rise of plasma motilin (89 ± 10 pmol/l at 50 min, $p < 0.05$), compared to the 30 min, prebreakfast value. The corresponding value during motilin was significantly higher at 126 ± 10 pmol/l. Following the infusion of the higher dose (0.68 pmol/Kg/min), levels rose further to 170 ± 11 pmol/l at 80 min, which was significantly higher ($p < 0.001$) than the corresponding control value of 63 ± 8 pmol/l.

Gastric emptying rate. The percentage of the radioactive meal marker, emptying from the stomach area, plotted against time is shown in Figure 502. Thirty minutes after the ingestion of the meal, and at the end of the lower motilin dose, $31 \pm 4\%$ of the marker had left the stomach area, whereas only $17 \pm 2\%$ had emptied during the control saline ($p < 0.01$). At the end of the higher motilin dose, $56 \pm 5.5\%$ of the marker had left the stomach as opposed to $40 \pm 5\%$ during the saline infusion ($p < 0.02$). Figure 503 shows the gastric half emptying time after motilin, in the individual subjects, expressed as a percentage of the corresponding saline control.

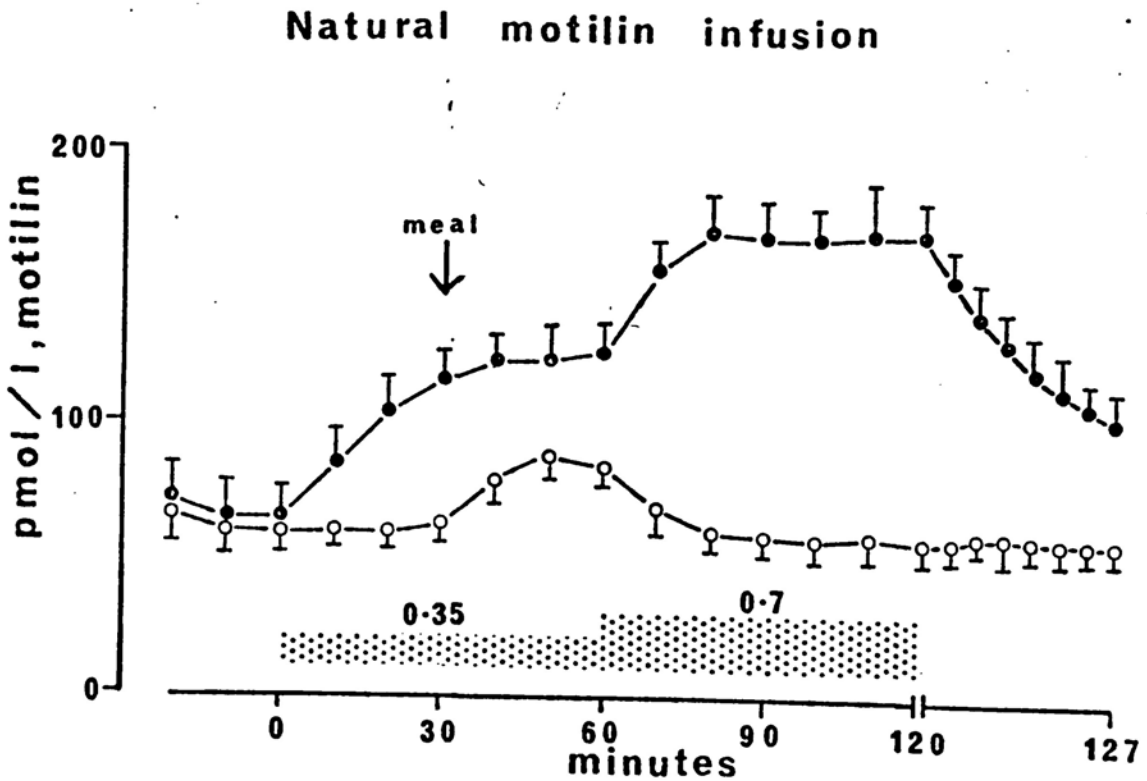


Figure 501

Plasma motilin levels (pmol/l) following ingestion of the meal during a saline infusion (open circles), or during motilin infusion (solid dots).

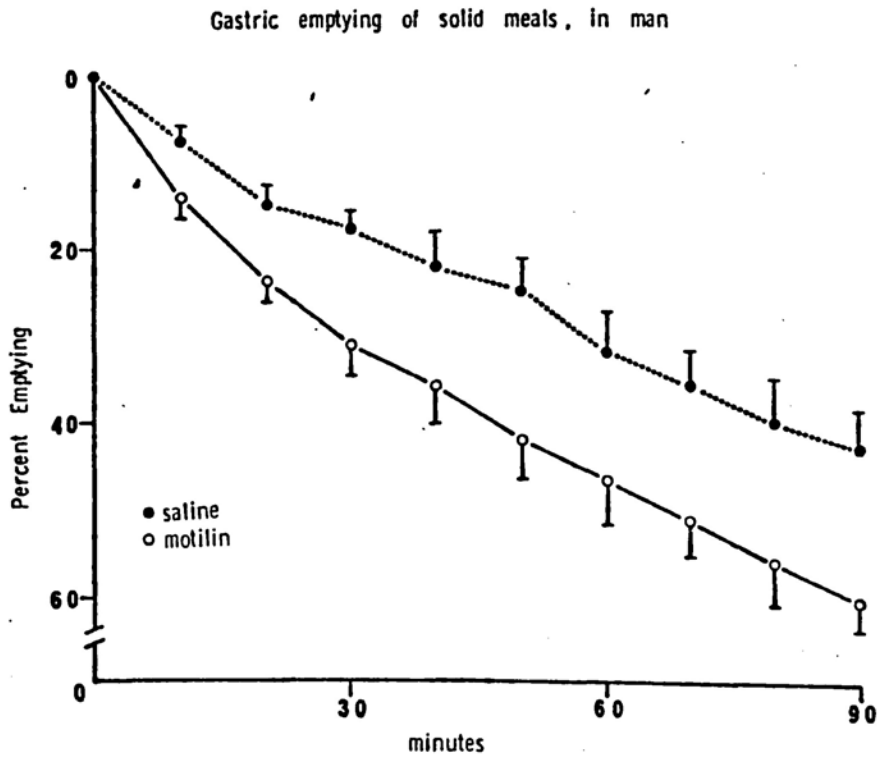


Figure 502

Percentage of the isotope emptying from the stomach during saline infusion (solid dots) and during motilin infusion (open circles).

EFFECT OF MOTILIN ON GASTRIC EMPTYING
OF SOLIDS IN MAN

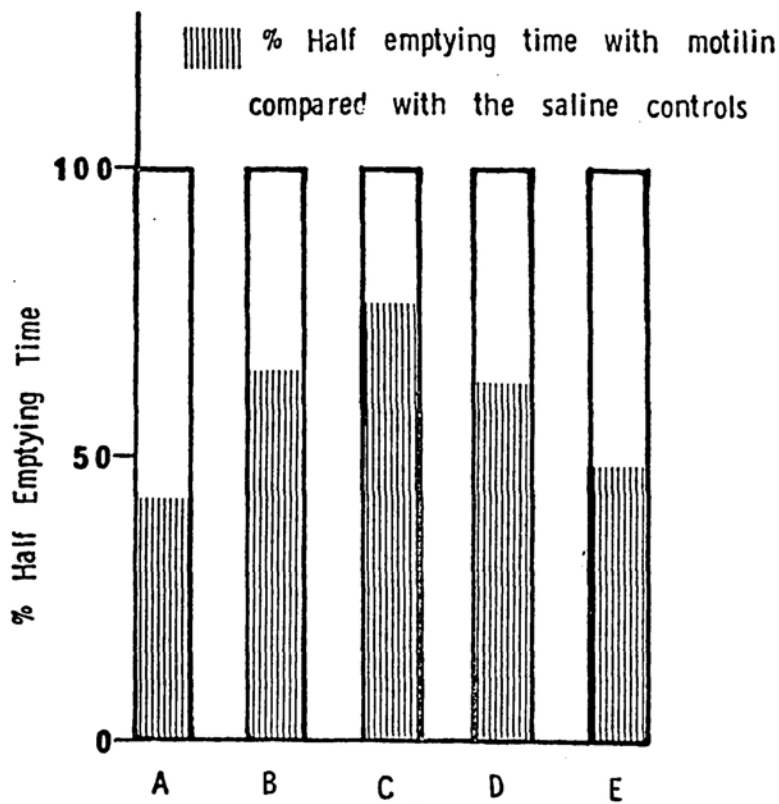


Figure 503

Half-emptying time with motilin (shaded area) expressed as a percentage of the emptying time with saline (clear area).

Pharmacokinetics. Figure 504 shows the post infusion values expressed as a percentage of the steady state plateau level plotted semilogarithmically against time (correlation coefficient 0.97). The $t_{1/2}$ was calculated to be 4.5 ± 0.4 min, the MCR (high dose) was 6.7 ± 0.7 ml/Kg/mins and the apparent distribution space was 44 ± 4 ml/Kg.

Effect of motilin on circulating hormones and plasma glucose

The effect of motilin on the release of various gut and pancreatic hormones is shown in Table 501. Infusion of saline or motilin did not significantly alter the fasting level of any of the hormones studied. Ingestion of the meal caused the release of insulin, GLP, PP and gastrin but there was no significant change in the plasma concentrations of entero-glucagon, VIP or pancreatic glucagon. The postprandial rise of insulin during motilin infusion was significantly higher ($p < 0.05$) than the rise seen during the saline infusion. The mean postprandial rise of GLP, PP and plasma glucose were also higher during the motilin infusion but these changes, however, did not reach statistical significance.

Gastric emptying of glucose and fat

Motilin levels in 'Glucose' study. The plasma motilin levels achieved in the glucose study are shown in Figure 505. On the control day, the mean zero time motilin concentration was 29 ± 7 pmol/l and this value was not significantly different from the mean pre-motilin infusion zero time value of 23 ± 5 pmol/l. There was no significant change in plasma motilin following 20 min of saline infusion. However, following infusion of motilin, plasma levels rose significantly ($p < 0.02$) to 44 ± 9 pmol/l, 20 min following the start of the infusion. Sixty min following ingestion of glucose, in the placebo, plasma motilin levels fell to 21 ± 4 pmol/l, though this was not statistically significant. This concentration was, however, significantly lower ($p < 0.01$) than the corresponding level of 59 ± 11 pmol/l achieved during the motilin infusion.

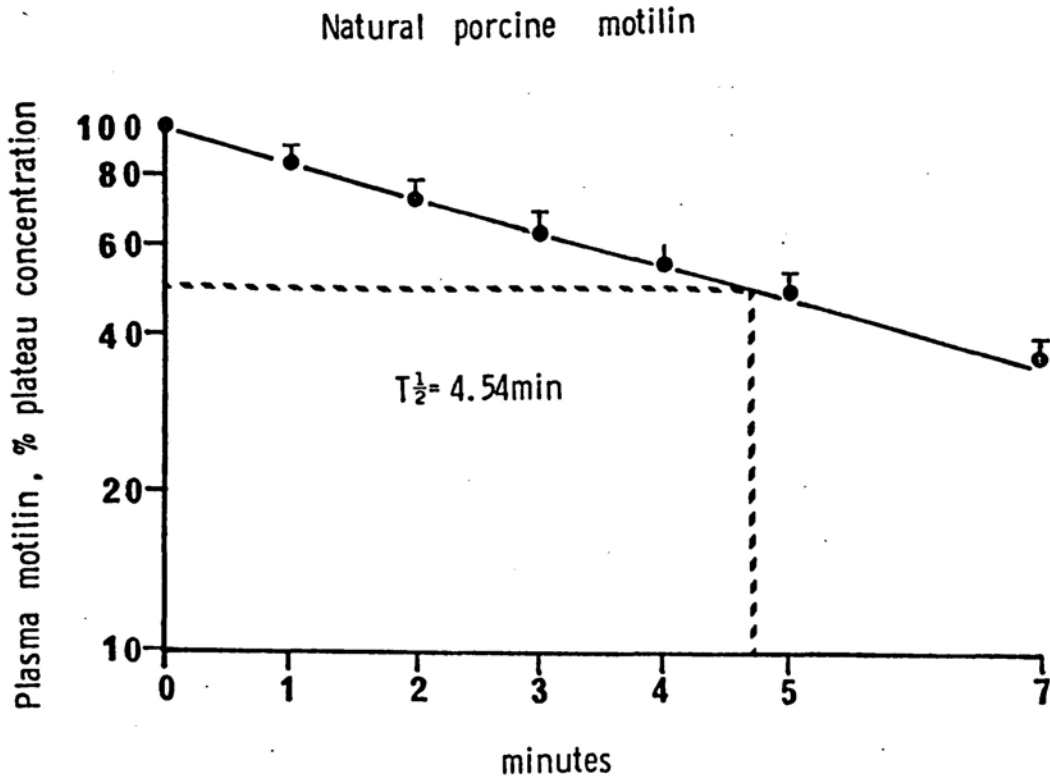


Figure 504

Post infusion motilin values expressed as a percentage of the steady state plateau level plotted semilogarithmically against time.

	S A L I N E				M O T I L I N			
	Pre-infusion Zero	30'	60'	120	Pre-infusion Zero	30'	60'	120'
Insulin	19±10	17± 8	190± 8	131±16	27±12	18± 9	330±45*	184±32
GIP	14± 4	15± 3	27± 5	32± 4	14± 4	14± 3	31± 5	40± 6
PP	25± 5	27± 3	209±39	185±29	20± 1	33± 6	223±32	253±62
Enteroglucagon	42±10	41±10	43± 9	41±11	35± 8	37±10	43± 9	45± 8
PG	8± 3	9± 3	9± 2	8± 2	8± 2	7± 2	8± 2	6± 1
VIP	4± 1	5± 2	3± 1	4± 2	4± 2	5± 2	3± 2	4± 1
Gastrin	2± 1	2± 1	17± 9	15± 5	2± 1	2±0.5	16±15	14± 5
Plasma glucose	4.59±0.09	-	6.14±0.44	4.64±0.32	4.84±0.15	-	6.67±0.19	4.72±0.25

Table 501

Concentration of plasma hormones, mean ± SEM, in pmol/l and blood glucose (in mmol/l) following the ingestion of a test meal during a saline infusion and motilin infusion.

* p<0.05

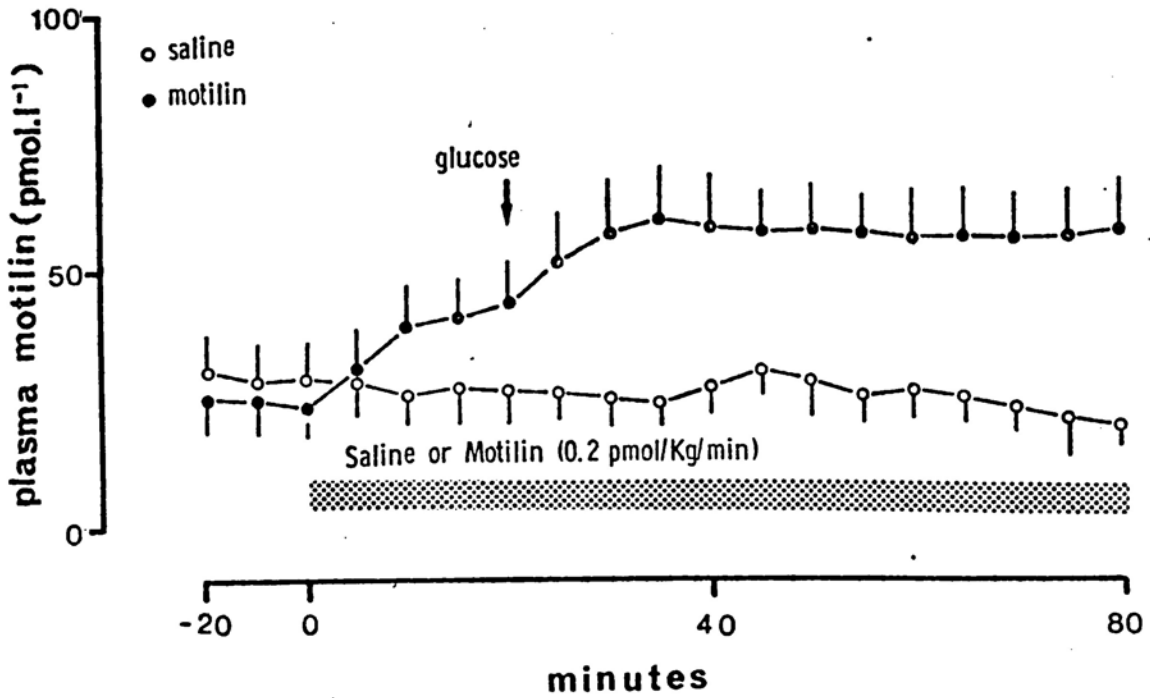


Figure 505

Plasma motilin levels (pmol/l) following glucose ingestion in the saline group (open circles) and during motilin infusion (solid dots).

Gastric emptying of glucose. Figure 506 shows the percentage of the radioactive marker emptying from the stomach area, plotted against time. Thirty minutes following ingestion of glucose during the motilin infusion, $25 \pm 2\%$ of the isotope had emptied, compared to only $11 \pm 1.5\%$ during placebo ($p < 0.005$). Sixty minutes following ingestion of glucose $44.5 \pm 3.5\%$ of the isotope had emptied compared to only $24.5 \pm 2.5\%$ in placebo ($p < 0.005$).

Effect of motilin on plasma insulin and glucose. The plasma insulin and glucose concentrations are shown in Figure 507. The pre-infusion zero insulin value in the placebo was 14.5 ± 5 pmol/l and on the motilin day it was 24 ± 5 pmol/l, (these were not statistically different). During the 20 min of the pre-meal infusion of both saline and motilin, there was no significant change in these concentrations. Ten minutes following ingestion of glucose plasma insulin levels rose to 48 ± 15 pmol/l in the placebo. This value was significantly lower ($p < 0.005$) than the corresponding insulin value on the motilin day (109 ± 17 pmol/l). Plasma insulin levels remained significantly higher during the motilin infusion at 20 and 30 minutes following ingestion of the glucose ($p < 0.01$).

The zero pre-infusion glucose value in the placebo was 3.7 ± 0.4 mmol/l which was not statistically different from the corresponding value of 3.8 ± 0.4 mmol/l on the motilin day. Twenty minutes of the pre-glucose saline or motilin infusion did not alter these concentrations in either group. Following ingestion of the glucose, plasma glucose concentrations rose to 5.5 ± 0.8 mmol/l and 6.4 ± 0.9 mmol/l at 20 and 40 minutes respectively in the control day. The corresponding plasma glucose concentrations during the infusion of motilin were 7.0 ± 0.6 mmol/l and 8.2 ± 0.8 mmol/l which were significantly higher than during placebo (both $p < 0.05$).

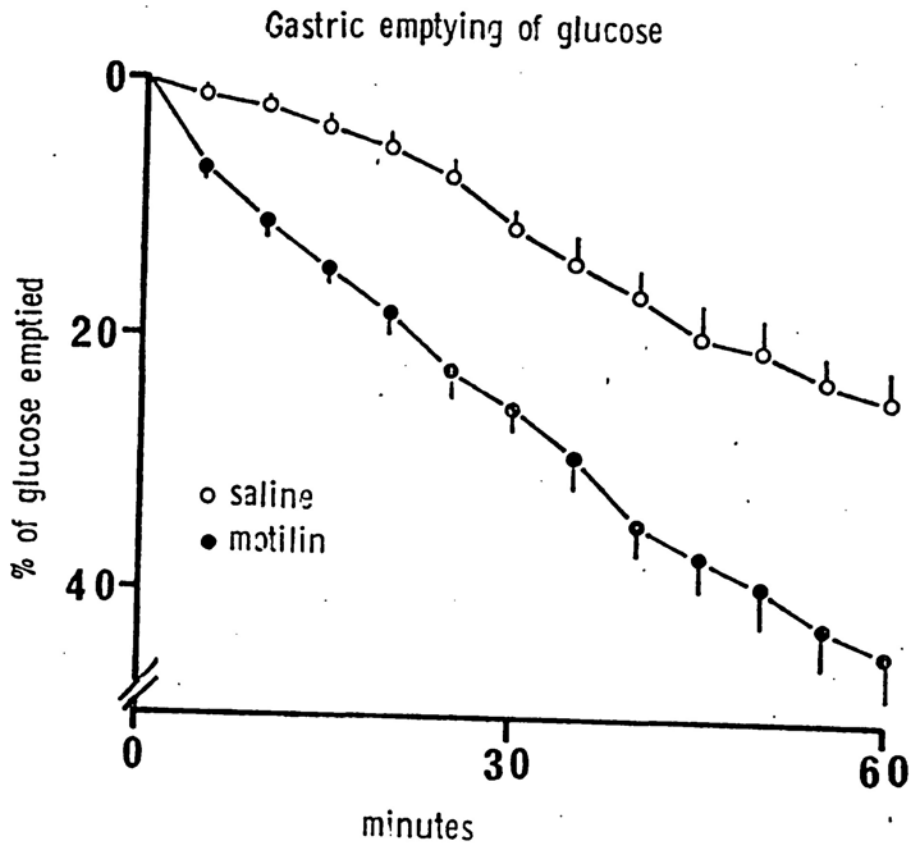


Figure 506

Percentage of the isotope emptying from the stomach during saline infusion (open circles) or motilin (solid dots) following glucose ingestion.

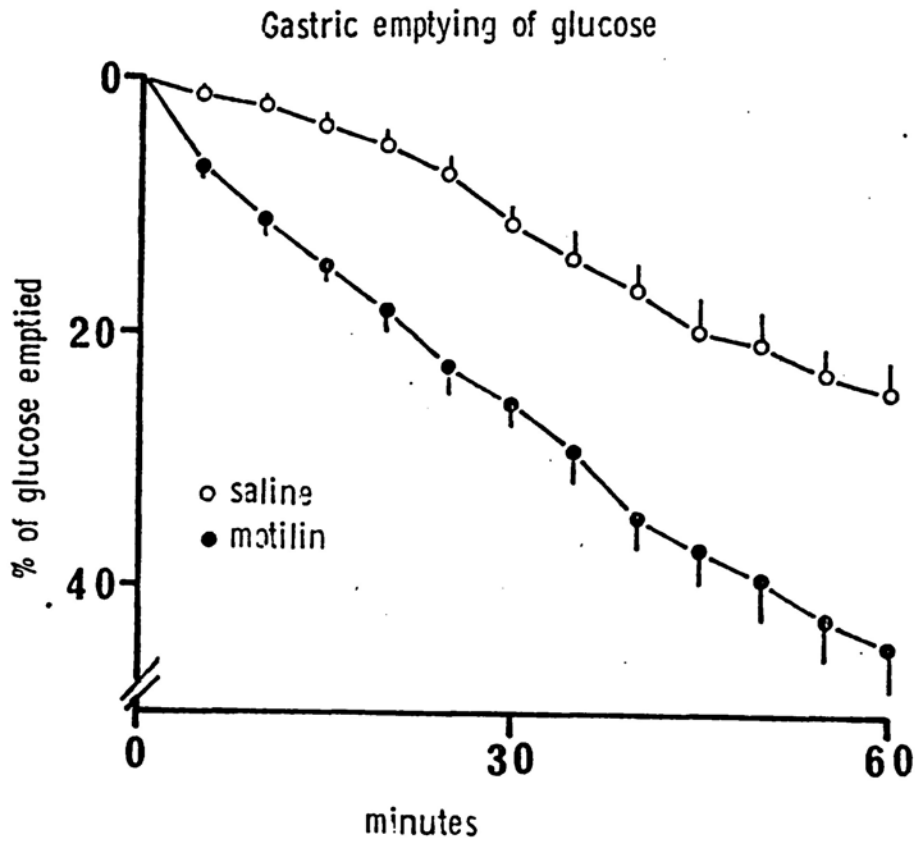


Figure 506

Percentage of the isotope emptying from the stomach during saline infusion (open circles) or motilin (solid dots) following glucose ingestion.

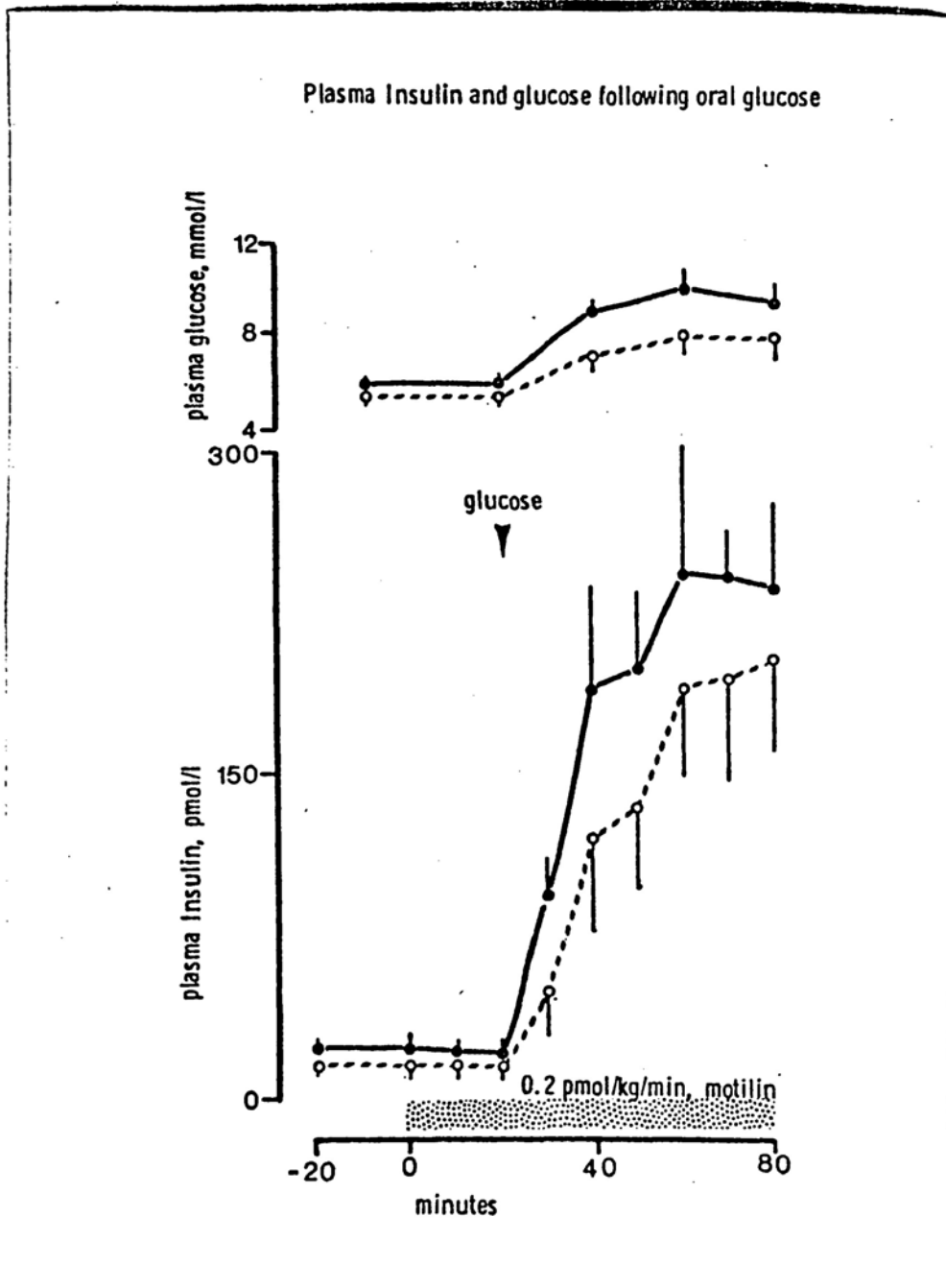


Figure 507

Plasma insulin levels (pmol/l, bottom panel) and plasma glucose (mmol/l, top panel) following glucose ingestion, during saline (broken lines) or motilin infusion (solid lines).

In vitro validation. The in vitro validation of the gastric emptying of fat showed that over 90% of the technetium $^{99\text{M}}$ tin colloid was distributed in the fat layer.

Motilin levels in 'fat' study. The plasma motilin levels achieved following ingestion of the double cream are shown in Figure 508. In the placebo, the mean zero time motilin concentration was $36 \pm 18 \text{ pmol/l}$ and this value was not significantly different from the mean pre-motilin infusion zero value of $40 \pm 11 \text{ pmol/l}$. There was no significant change in plasma motilin following the 20 minutes pre-cream saline infusion. However, motilin levels rose significantly ($p < 0.02$) to $62 \pm 9 \text{ pmol/l}$ at 20 min following the pre-cream infusion of motilin. Thirty minutes following ingestion of the cream, in the placebo, motilin levels rose to a peak of $84 \pm 12 \text{ pmol/l}$ ($p < 0.01$). This value was similar to the corresponding peak level of $80 \pm 13 \text{ pmol/l}$ achieved during the motilin infusion.

Gastric emptying of fat. Figure 509 shows the percentage of the radioactive meal (cream) marker remaining the stomach area, plotted against time. Thirty minutes following ingestion of the cream, $17 \pm 2\%$ had emptied in the saline controls which was not different from the corresponding level of $14 \pm 3\%$ during infusion of motilin. At no stage during the experiment was there any significant difference between the two days.

Plasma chromatography. The chromatographic profile of plasma motilin immunoreactivity is shown in Figure 510. In the basal state two immunoreactive motilin peaks were detected, one eluting with an elution constant of 0.16 (peak I) and the other coeluting with the porcine motilin marker ($K_{av} = 0.67$) (peak II). In the placebo (top panel) ingestion of cream resulted in an increase of both motilin peaks (peak I rose by $95 \pm 31\%$, $p < 0.05$ and peak II by $202 \pm 42\%$, $p < 0.01$). Ingestion of the cream, during motilin infusion (bottom panel) resulted in a rise of peak II ($395 \pm 69\%$, $p < 0.005$) but a reduction of peak I ($-42 \pm 7\%$, $p < 0.005$), (Tables 502 and 503).

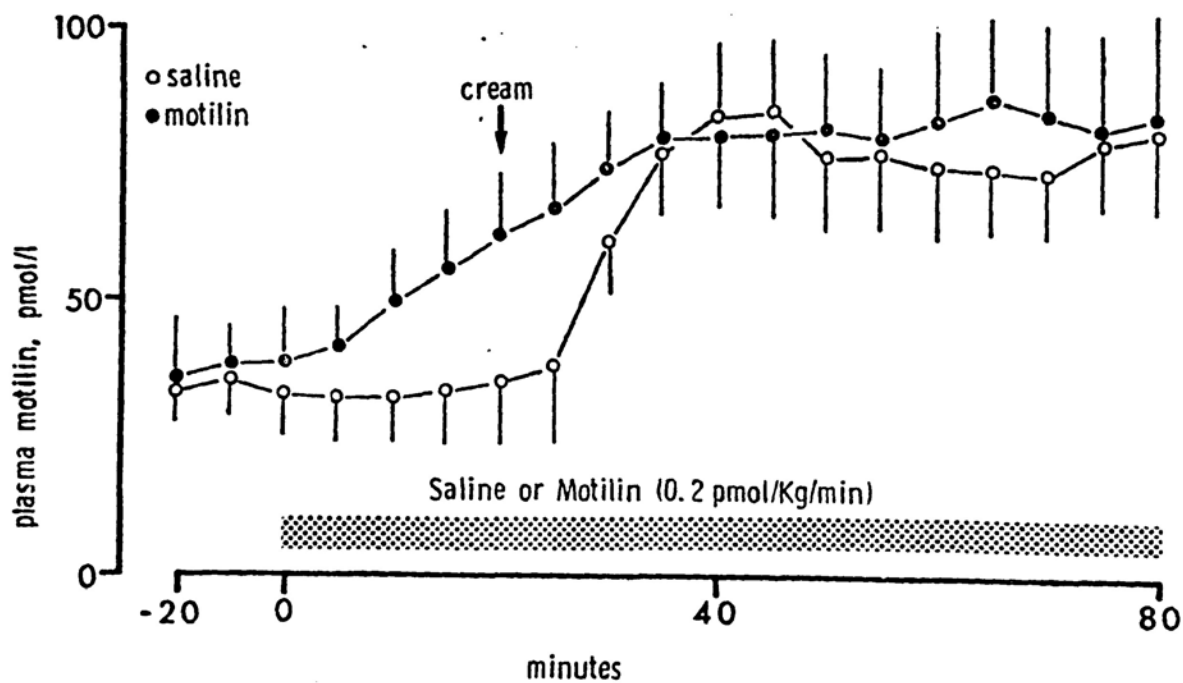


Figure 508

Plasma motilin (pmol/l) following the double cream ingestion during saline (open circles) or motilin infusion (solid dots).

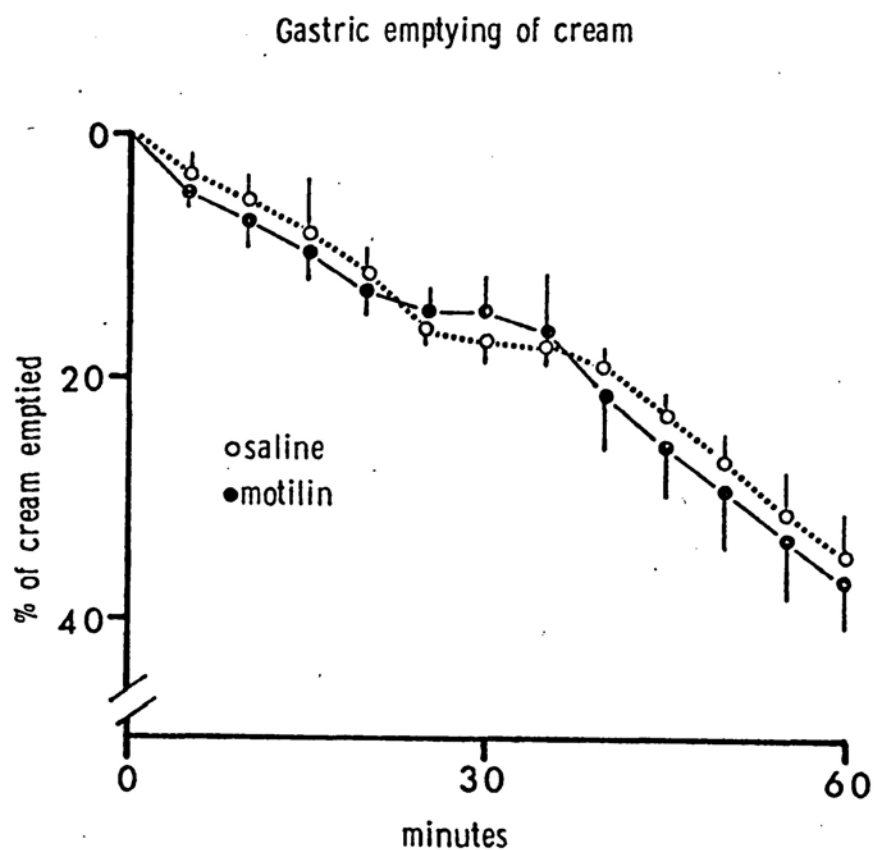


Figure 509

Percentage of the isotope emptying from the stomach during saline (open circles) or motilin infusion (solid dots) following cream ingestion.

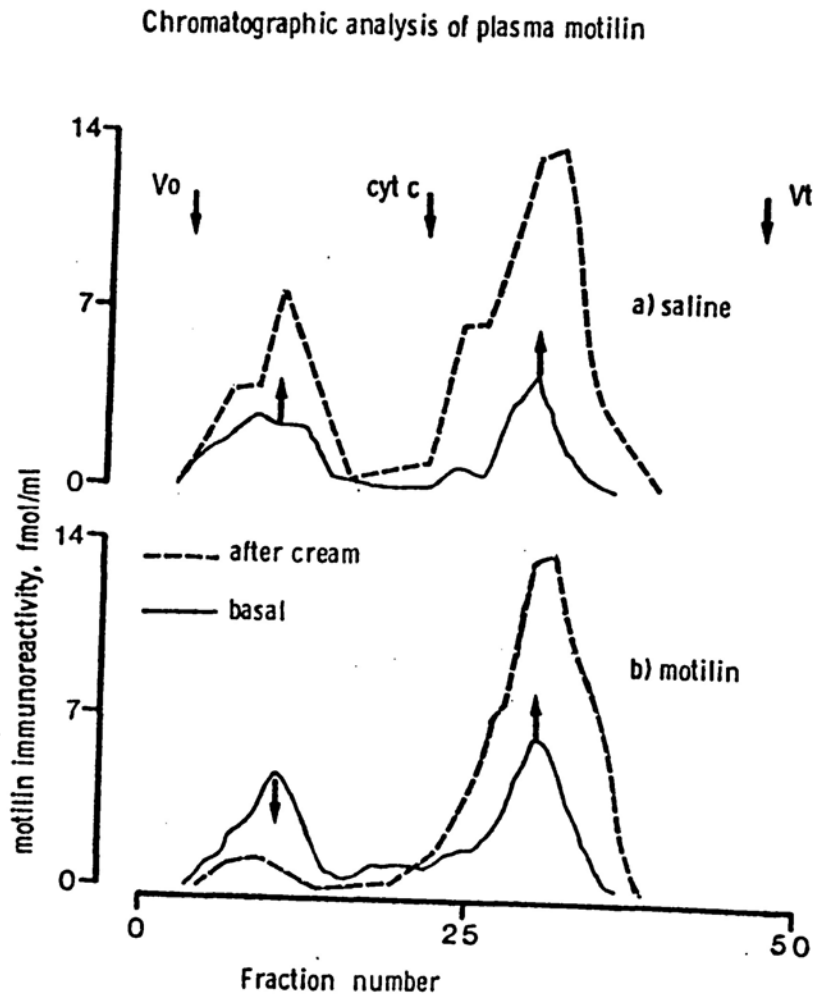


Figure 510

Gel chromatography elution profile of motilin immunoreactivity in fasting plasma (solid lines) and following double cream ingestion (broken lines) during saline (top panel) or motilin infusion (bottom panel).

CREAM (PLACEBO)

<u>Peak I</u>				<u>Peak II</u>		
<u>Sub</u>	<u>Basal</u>	<u>stimulated</u>	<u>% change</u>	<u>Basal</u>	<u>stimulated</u>	<u>% change</u>
1	12	24	100	25	52.5	110
2	9	27	200	17	41	140
3	14	25	79	12	45	275
4	18	27	50	21	85	305
5	20	29	45	16	45	181
mean	14.6	26.4	94.8	18.2	53.7	202.2
$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
SEM	2.2	0.95	31.5	2.5	9	42.3

Table 502

Plasma motilin immunoreactivity (fmol/l) following gel permeation analysis in 5 subjects following ingestion of cream, during a saline infusion.

CREAM AND MOTILIN INFUSION

<u>Sub</u>	<u>Peak I</u>			<u>Peak II</u>		
	<u>Basal</u>	<u>stimulated</u>	<u>% change</u>	<u>Basal</u>	<u>stimulated</u>	<u>% change</u>
1	15	7	-55	18	80	344
2	13	9	-33	13	78	497
3	22	16	-27	25	64	255
4	15	10	-33	8	54.5	580
5	21	8	-60	25	100	300
mean	17.2	10	-41.6	17.8	80.7	395.2
$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
SEM	2	1.8	7.4	3.7	8.9	68.9

Table 503

Plasma motilin immunoreactivity (fmol/ml) following gel permeation analysis in 5 subjects following ingestion of cream, during a motilin infusion.

3. Motilin and the interdigestive migration motor complex in man

The various phases of the interdigestive motor complex could be easily identified in all tracings but the most striking feature was the burst of rhythmic contractions called the activity front (86), (Figure 511). The average time interval between fronts was 137 ± 22 min. Fasting motilin levels varied between different subjects, and also varied with time in each subject (Table 504). These fluctuations, however, did not occur at random but were related to the different phases of the interdigestive motor complex (Figure 512). The mean motilin level of the 30-min period preceding the appearance of the activity front in the gastroduodenal area was significantly higher than the mean level for the 30-min period that followed the activity front ($p < 0.001$). The highest level occurred some 15 min prior to the onset of the activity front at the level of the angle of Treitz and was 13 pmol/l higher than the lowest concentration in the quiescent period ($p < 0.001$).

The intravenous infusion of porcine motilin was able to induce activity fronts. At a nominal infusion rate of 0.4 pmol/Kg/min a front was induced in 1 of the 3 subjects, and an infusion rate of 0.8 pmol/Kg/min induced a front in 2 out of 4 subjects. Infusion rates of $1.6\text{--}6.4 \text{ pmol/Kg/min}$ were invariably followed by an activity front (Table 505). The mean interval between the preceding spontaneous occurring activity front and the activity front that followed the motilin infusion was 46 ± 3.5 min, which was significantly shorter than the time interval between spontaneously occurring fronts (137 ± 22 mins, $p < 0.001$). The characteristics of the motilin-induced activity fronts were not different from the characteristics of the spontaneous occurring fronts (Table 506).

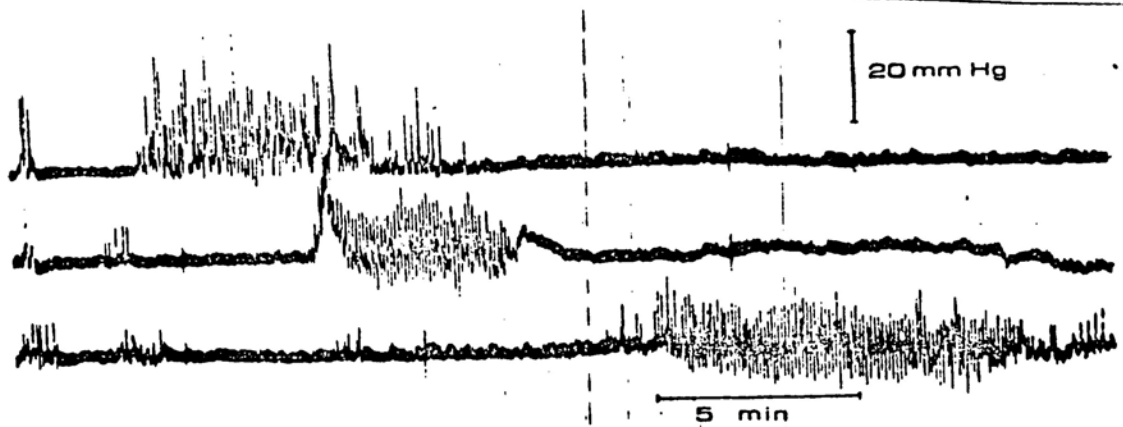


Figure 511

The activity front of the interdigestive motor complex is characterised on manometric tracings by a burst of rhythmic contraction waves that progresses down the intestine.

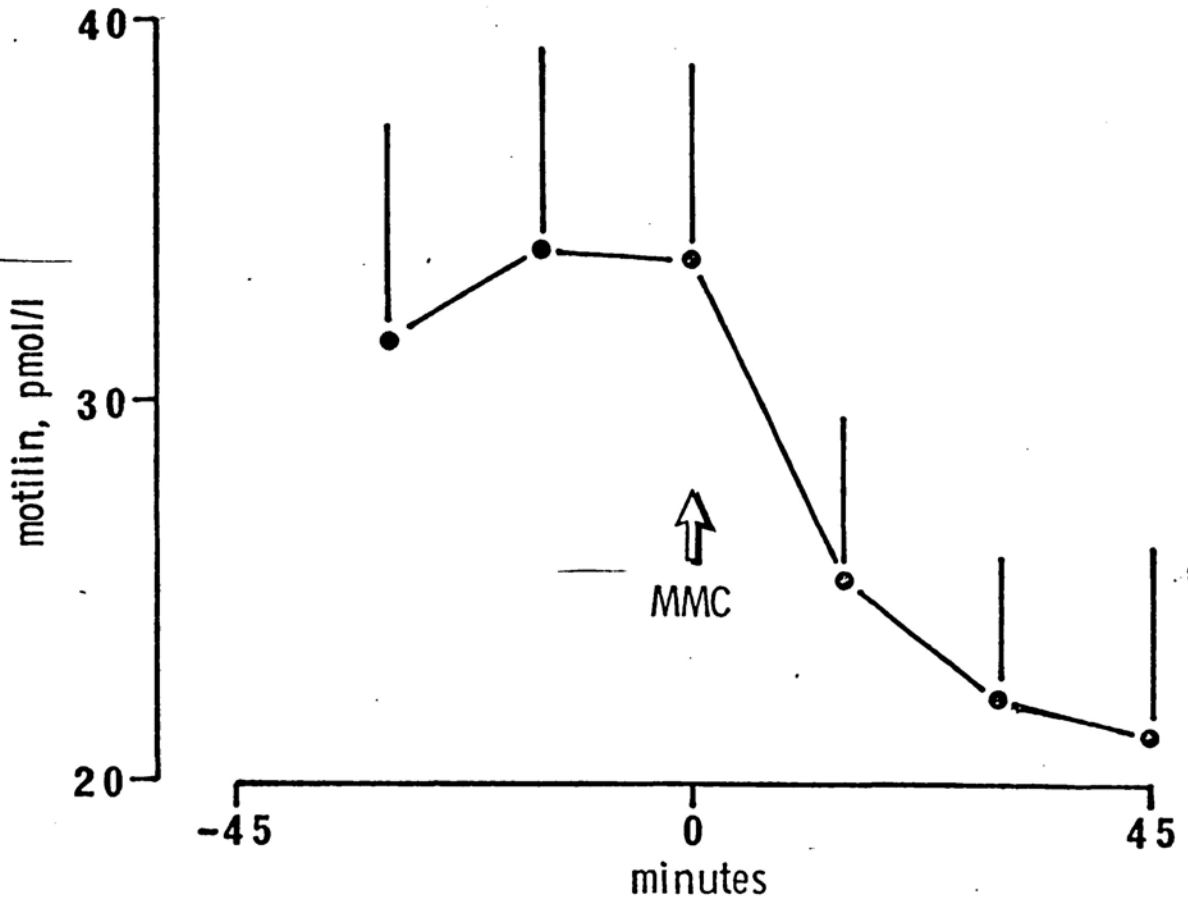


Figure 512

Plasma motilin in 23 healthy subjects during an MMC. Time 0 indicates the onset of an activity front at the level of the angle of Treitz.

Sub	-30	-15	0	15	30	45
1	14	19	26	22	20	10
2	14	19	29	26	23	10
3	10	12	12	11	8	6
4	13	24	24	13	13	7
5	14	20	15	28	21	8
6	26	26	27	20	21	14
7	28	34	33	20	23	7
8	30	31	25	21	24	23
9	73	77	63	33	36	39
10	38	34	35	35	16	8
11	33	29	22	22	26	30
12	86	87	67	42	24	19
13	20	22	22	18	17	20
14	16	16	15	9	10	13
15	8	11	13	8	10	13
16	27	42	42	36	26	28
17	27	21	15	17	23	31
18	15	17	15	5	5	5
19	38	34	35	22	20	17
20	114	113	113	103	93	115
21	44	58	80	56	38	38
22	17	26	33	16	11	8
23	23	30	29	13	5	5
X	31.6	34	33.8	25.5	22.3	21.3
SEM	5.6	5.3	5.05	4.22	3.77	5.1

Table 504

Plasma motilin (pmol/l) in 23 healthy subjects during an MMC. Time 0 indicates the onset of an activity front at the level of the angle of Treitz.

Infusion rate (pmol/Kg/min)	Plasma motilin rise (pmol/l)* (Mean $\pm$ SEM)	Activity front response**	Interval between spontaneous and induced front (min) (Mean $\pm$ SEM)
0.4	46 $\pm$ 7	1/3	42
0.8	107 $\pm$ 22	2/4	36, 54
1.6	125 $\pm$ 19	3/3	51.3 $\pm$ 4.7
3.2	200 $\pm$ 49	4/4	53.2 $\pm$ 4.4
6.4	351 $\pm$ 40	2/2	43, 41

Table 505

Induction of activity front by motilin infusion.

* Computed as the difference between the peak motilin value during infusion and the last motilin value available before the onset of the infusion. ** Number of subjects in whom an activity front was induced/number of subjects tested.

	Spontaneous activity fronts			Motilin induced activity fronts		
	Duod*	Jej I**	Jej II***	Duod	Jej I	Jej II
Duration (min)	3.7±0.7	4.1±0.3	4.5±0.4	4.4±0.4	4.8±0.3	5.3±0.4
Frequency of contractions (N/min)	11.6±0.3	11.8±0.1	11.6±0.2	11.7±0.2	11.6±0.1	11.3±0.1
Progression velocity (cm/min)	10.1±1.8	6.8± 0.9		8.2±1.1	6.0±0.6	
Calculated length (cm)		18.8±3.3.			18.8±2.9	

Table 506

Characteristics of spontaneous and motilin-induced activity fronts in the same subjects,
 *Duod : duodenum, **Jej I : 15cm below angle of Treitz, ***Jej II : 40cm below angle of Treitz.

DISCUSSION

1. Gastric emptying

Since the time of Beaumont (122) it has been appreciated that the mechanisms controlling gastric emptying are very complex. Most factors that influence gastric emptying do so by inhibiting (such as fats (123), acids (124) and hyperosmolar (125) solutions), to varying degrees, the action of the gastric pump. Distension of the stomach is the only natural stimulus known to increase gastric emptying (126) by exciting the gastric pump to greater activity. Because distension is the most potent stimulus for motilin release and because of the stimulatory action of motilin on gastrointestinal smooth muscle, experiments were undertaken to investigate the effect of motilin on gastric emptying of various substances.

The emotional state of the subjects has an important general influence on the rate of gastric emptying. In states of fear, for example, food may remain in the stomach for 12 hours or more, whereas excitement may reduce emptying time (127). In order to randomise any possible psychological influence of a forthcoming infusion, the subjects were not told of the nature of the infusion they received. No unexpected effects were observed during the infusions, such as change of pulse rate, nausea, etc., and there was no apparent effect on appetite or the rate of food consumption. The rate of gastric emptying was measured by an established method employing ^{129}Cs or $\text{Tc}^{99\text{M}}$ as the isotope marker. In vitro validation experiments revealed that the Cs^{129} was distributed in the solid layer of the mixed breakfast and that the technetium $^{99\text{M}}$ tin colloid was distributed in the fat layer. The data presented here show that exogenous infusions of porcine motilin accelerate the gastric emptying of a mixed meal and of glucose and this results in higher postprandial levels of insulin and glucose. In view of the suppression of motilin by intravenous glucose it is tempting to speculate the existence of a negative

feedback mechanism with glucose controlling its own absorption, via its effect on motilin and the subsequent changes in gastric motility. The effect of motilin on insulin and glucose appears to be indirect (via the acceleration of gastric emptying), as infusions of motilin during the basal, pre-meal state did not influence insulin and glucose levels. The effect of motilin on the gastric emptying of fat, however, appears to be more complex. Infusions of motilin, at the doses used, appeared to be ineffective in altering the gastric emptying of fat. Subsequent measurement of motilin revealed that the levels were not significantly different during motilin infusion when compared with the placebo. A possible explanation for this paradox was provided by the gel permeation analysis of the plasmas. In the basal state two immunoreactive motilin peaks were detected. One peak co-eluted with the pure porcine motilin standard, whereas the other peak eluted earlier in a position suggesting a much higher molecular weight substance. Fat ingestion during the saline infusion resulted in a rise in both immunoreactive motilin peaks. In contrast, fat ingestion during infusion of motilin was accompanied by the expected rise in the latter peak but a suppression of the earlier eluting peak I, a similar situation shown earlier (Chapter 4), during the interdigestive state. Further studies on the gastric emptying of fat, using higher motilin doses are now clearly required.

The only other published work of the effect of motilin on gastric emptying, in man, is the report of Ruppin and colleagues (38) showing that motilin in 3 subjects, delayed the gastric emptying of a solution containing 10% mannitol in 0.5M citric acid phosphate buffer pH3.0. However, Ruppin's study is not strictly comparable with the present one, as the dose and nature of motilin (they used 13-Nle-motilin) employed was different (3-12 times higher). Preliminary work in our laboratory, employing higher doses, similar to those used by Ruppin et al, suggest that motilin can, under these circumstances, inhibit the gastric emptying of glucose. Motilin infusions in

other species, rat (40) and dog (39), were able to cause acceleration of the gastric emptying of saline, thus confirming the results presented here. However, it was also reported, that motilin had no effect on the gastric emptying of solids, in the dog (39). The discrepancy between these results and the present can perhaps be explained by the different methodologies used and also more importantly, species differences.

As mentioned earlier, fat and acid are potent inhibitors of gastric emptying and yet both these factors provoke a release of plasma motilin (shown here to stimulate gastric emptying). A possible explanation for this apparent discrepancy, may be that, administration of fat and acid may stimulate the release of inhibitory hormones which may have an overriding effect on motilin and thus inhibit gastric emptying. A large number of such inhibitors have been put forward over the years, such as cholecystokinin (128, 129) and gastrin (130, 131). Possible future research may involve the simultaneous infusion of many hormones and studying their effects on gastric emptying.

Certainly motilin appears to fulfill some of the criteria of a physiological hormone controlling gastric emptying i.e. it is released following a stimulus known to accelerate gastric emptying, (gastric distension) and when endogenous motilin levels are mimicked by an exogenous infusion the same phenomenon is observed.

2. Pharmacokinetics

During the infusion of 13-Nle-motilin at doses of 0.6 and 2.4 pmol/Kg/min for 40 min in man, plasma motilin rose to a plateau of 124 and 360 pmol/l, respectively, and fell rapidly afterwards with first order kinetics and a half-life of 4.4 min. The calculated metabolic clearance rate and apparent distribution space were 7.8 ml/Kg/min and 49.4 ml/Kg, respectively (132). The half life of endogenous motilin was determined in man by following the changes in plasma concentration after administration of somatostatin and was found to be 4.6 min (132). The infusion of natural porcine motilin (the results presented here) at doses of 0.34 and 0.68 pmol/Kg/min resulted in plasma

motilin increments of 58 and 102pmol/l. The half life was calculated to be 4.5 min, the metabolic clearance rate was 6.7 ml/Kg/min and the apparent distribution space was 44ml/Kg (all the above studies were carried out using antibody M1). Thus the pharmacokinetics in man of natural porcine motilin, 13-Nle-motilin and endogenous human motilin are very similar. These results however are not strictly accurate as the inhibition of peak I motilin by the exogenous motilin infusion was not considered and must await further studies employing an antibody that does not crossreact with peak I motilin or using chromatographic analysis.

3. Motilin and the interdigestive migrating motor complex in man

In 1969, Szurszewski (113) identified in the small intestine of fasted dogs an electric complex characterised by a front of intense spiking activity that migrated down the entire small bowel. As the complex reached the ileum, another activity front developed in the duodenum. Feeding interrupted this cycle and changed the tracing into a pattern of irregular spiking activity (34). Code and Marlett (33) divided the complex in four different phases. Phase one is characterised by the relative absence of action potentials; during phase two there is persistent but irregular action potential activity; phase three, the activity front, is characterised by its sudden onset and end, and by the presence of large action potentials; during phase four, incidence and intensity of action potentials decline rapidly and disappear completely. The complex has since been described in several species and its motor equivalent in man has been described recently by Vantrappen and colleagues (86). The mechanisms that control the interdigestive motor complex is poorly understood. Both neural (134, 135, 136) and hormonal factors (134, 135) seem to be involved. Vagotomy produces disorders of the motor complex (135,136) whereas in an isolated but innervated intestinal loop the front occurs in a normal sequence with the other parts of the

small intestine (137, 138). High doses of gastrin (135) and insulin interrupt (139) the complex, but patients with hypergastrinemia (Zollinger-Ellison syndrome and pernicious anaemia) have normal complexes (140), and physiological doses of pentagastrin do not eliminate the activity front in normal subjects (141).

Several investigators have recently suggested that motilin may have a role in the control of interdigestive motor activity (34,35, 134). They showed, in dogs, that the appearance of the activity front in the gastroduodenal area was associated with a significant rise in plasma motilin levels and that infusion of synthetic motilin induced migrating bursts of small bowel contractions that closely resemble the activity front. The data presented here have now shown that similar results can also be obtained in man.

A statistical significant rise in serum motilin preceded the initiation of the activity front of the interdigestive motor complex in the gastroduodenal area. Also exogenous infusions of porcine motilin induced premature activity fronts that ~~were~~ not different from the spontaneous activity fronts.

In contrast to the above studies, Borody and colleagues (142) reported, in a preliminary communication, that in the pig, plasma motilin did not differ in any identifiable causal manner in association with the migrating motor complex (MMC). The same investigators also reported, that infusions of natural and synthetic 13-Nle-motilin, failed to provoke, prematurely, the onset of M.M.C.s which continued at their usual frequency (reported to be 55 min, which is about half than that in the dog and man).

The increment in plasma motilin, needed to induce an activity front by intravenous infusion of porcine motilin, was higher than the rise in endogenous motilin preceding a spontaneous activity front. Although this may be partly due to the timing of the infusion ie a period of the complex more refractory to motilin or due to the use of motilin from a different species (porcine) that may be less active than

human, it could also be that motilin is not of prime importance in the initiation of M.C.C.s. Perhaps in accord with the latter hypothesis is the finding of Lux and colleagues (143) of somatostatin induced MMCs, in man. Thus, it was shown that although motilin levels were inhibited by somatostatin a premature duodenal complex was still initiated, suggesting that motilin is not the main control mechanism. Poitras and colleagues (144) also showed, in the dog, that somatostatin initiated an MMC although this began in the jejunum.

Thus although plasma motilin has an identifiable causal association with MMCs and exogenous motilin infusion can initiate new fronts, a physiological role of motilin has not yet been proven.

CHAPTER 6
MOTILIN AND GASTROINTESTINAL PATHOLOGY

Introduction

Although the physiology of motilin is by no means established, the data obtained to date, suggest a role of motilin in the control of gastrointestinal motility. There are a large number of diseases in which motor disorders of the gastrointestinal tract have been described, therefore a preliminary screen of such diseases was undertaken.

Methods

Standard test breakfast

As a stimulus for motilin release, a test breakfast was used consisting of two medium sized boiled eggs, 60g bread as toast, 10g butter, 35g marmalade and 150 ml unsweetened orange juice. The breakfast which contained 18g protein, 22g fat and 66g carbohydrate (530 Calories) was eaten by all subjects following an overnight fast.

Patients

Brief clinical details of all patients studied including age, sex and weight are shown in Table 601.

	Number of Subjects		Age	Weight (Kg)
	M	F	Mean(range)	(meant $\pm$ SEM)
Tropical Sprue	4	4	29(23-49)	58 $\pm$ 3
Infective diarrhoea	7	5	37(19-67)	64 $\pm$ 3
Crohn's disease	7	7	35(13-70)	49 $\pm$ 4
Ulcerative Colitis	10	14	38(14-74)	59 $\pm$ 3
Diverticular disease	3	9	63(43-78)	60 $\pm$ 4
Irritable bowel disease with diarrhoea	3	16	42(20-77)	71 $\pm$ 4
Irritable bowel disease with constipation	3	8	47(21-87)	70 $\pm$ 3
Small Intestinal Resection	10	8	48(20-72)	54 $\pm$ 3
Large Intestinal Resection	4	5	34(21-62)	53 $\pm$ 4
Controls	23	8	39(23-62)	67 $\pm$ 5

Table 601

Details of number, sex, age and weight of the patients investigated.

'Infective' diarrhoea

Twelve patients, five women and seven men aged 37 years (range 19-67 years) were studied. All had previously been in good health and none had a history of gastrointestinal disease. They had all required admission to hospital for severe diarrhoea (at least 8 motions per day) and had been treated with intravenous fluid and electrolyte replacement. Probable pathogens were isolated from four patients. No probable pathogens were cultured from the remaining eight patients, nor were any ova, cysts or parasites observed on microscopy. Barium enema radiology was normal in each case. All patients made complete and lasting recoveries.

Tropical Malabsorption

Eight consecutive patients with severe tropical malabsorption presenting at the Hospital for Tropical Diseases were studied. Their selection was made on clinical and biochemical grounds. (Table 602).

Crohn's Disease

Fourteen patients with regional enteritis (Crohn's disease) were studied (7 male and 7 female, aged 35 years) (13-70 years) and mean weight of 49 ± 4 Kg). The patients typically presented with pain and diarrhoea and the diagnosis was made on radiological and histological evidence.

Subject	Sex	Age (Years)	Weight Loss (kg)	Length of History (months)	Mean Faecal Fat/24 hr (mmol/l)	Mean Urinary Xylose (mmol/5 hr)
1	M	26	15.9	3	ND	2.9
2	M	19	19.1	4	105	2.4
3	F	39	7.3	5	116	1.5
4	F	26	15.9	3	64	4.4
5	M	57	19.1	3	38	3.5
6	F	20	19.1	5	20	3.8
7	M	28	15.9	3	55	2.8
8	M	31	9.5	3	80	1.4
Mean	5 M 3 F	31	15.2	3.6	68.3	2.8
SEM or Range		19-57	1.6	0.3	13.1	0.4
Normal Values					11-18	8-16

Table 602

Clinical details of patients with Tropical Malabsorption.

Ulcerative Colitis

Twenty-four patients with ulcerative colitis were studied (10 male and 14 female, aged 38 years) (14-74 years), and mean weight of 59 ± 3 Kg). These patients presented with diarrhoea and the diagnosis was made by sigmoidoscopy together with histological evidence from colonic biopsies.

Diverticular Disease

Twelve patients with diverticular disease of the colon were studied (3 male and 9 female, aged 63 years) (43-78 years) and mean weight of 60 ± 4 Kg). The patients presented with a history of pain and diarrhoea and the diagnosis was confirmed radiologically with a barium enema.

Irritable Bowel Syndrome

Thirty patients with the irritable bowel syndrome were studied. Of these 19 patients complained primarily of pain and diarrhoea (3 male and 16 female, aged 42 years) (20-77 years) and mean weight 71 ± 4 Kg) and in 11 patients the disease was predominantly associated with constipation (3 male and 8 female, aged 47 years) (21-87 years) and mean weight of 70 ± 3 Kg).

Short Bowel Syndrome

Eighteen patients (10 male and 8 female, aged 48 years) (range 20-70 years) and mean weight of 54 ± 4 Kg) with partial ileal resection were studied together with 9 patients (4 male and 5 female, aged 34 years) (21-62 years) and mean weight of 53 ± 4 Kg) who had undergone partial colonic resection. Gut resection had been undertaken for inflammatory bowel disease, neoplasia, radiation, fibrosis or trauma, a minimum of one year prior to the study.

Controls

A group of 31 healthy subjects, described in Chapter 4 acted as controls.

RESULTS

'Infective' Diarrhoea

Patients with 'infective' diarrhoea had elevated basal motilin levels, 141 ± 26 pmol/l ($p < 0.001$) when compared to the controls, 48 ± 6.5 pmol/l (Figure 601 Table 603). Following ingestion of the breakfast, in the infective diarrhoea group, plasma motilin rose significantly to a peak at 15 min of 182 ± 29 pmol/l ($p < 0.005$ against basal and $p < 0.001$ against corresponding values in the controls, 71 ± 10 pmol/l).

The incremental rises (15 min value minus basal) however were not statistically significant in the two groups (42 ± 11 pmol/l in the infective diarrhoea group versus 25 ± 5.5 pmol/l in the controls).

Tropical Malabsorption

Basal plasma motilin levels in the eight patients with tropical malabsorption were significantly higher (142 ± 28 pmol/l, $p < 0.001$) than the corresponding control values (48 ± 6.5 pmol/l). The peak postprandial motilin release occurred later (at 30 min) when compared to the control group (15 min) (Table 604 and Figure 602). The 30 min value in the tropical malabsorption group was 184 ± 32 pmol/l which was significantly higher than the corresponding control value. The incremental rises, however, were not significantly different in the two groups.

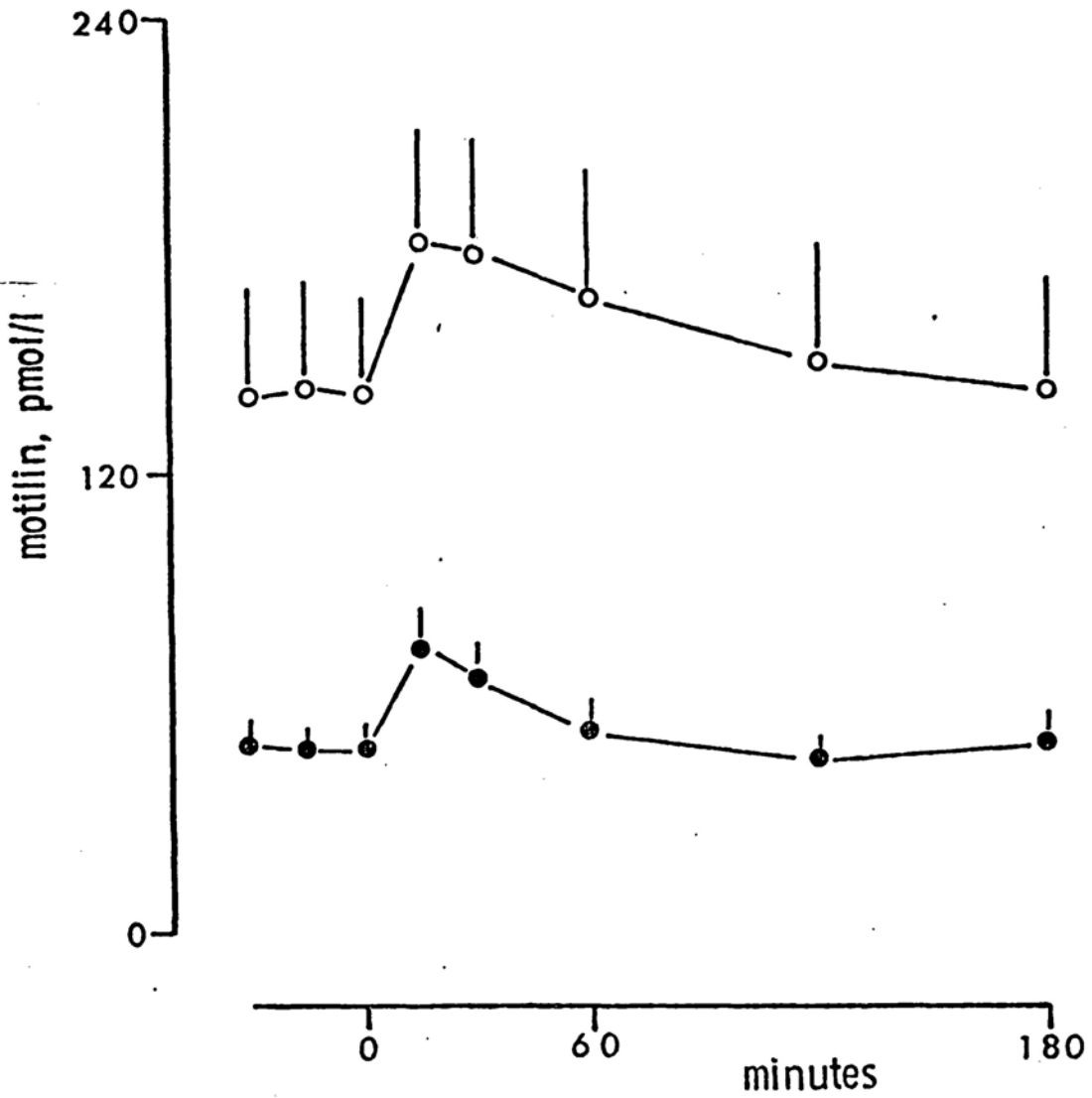


Figure 601

Plasma motilin (pmol/l) in patients with 'infective' diarrhoea (open circles) and controls. The meal was given at time zero.

Sub	-30	-15	0	15	30	60	120	180
1	40	32	50	122	72	56	72	68
2	24	20	24	64	46	24	24	28
3	69	52	60	120	98	72	46	36
4	214	164	164	232	216	304	290	240
5	124	184	184	176	174	126	124	96
6	244	346	316	400	352	336	280	292
7	128	128	142	228	260	216	202	210
8	188	206	182	170	194	142	120	110
9	212	220	214	280	308	334	314	284
10	52	98	82	100	114	104	56	60
11	52	36	32	56	36	28	36	34
12	340	224	236	240	264	254	228	252
X	141	143	141	182	178	166	149	142
SEM	28	28	26	29	30	34	31	30
<u>log transformation</u>								
<u>of raw data</u>								
X	2.02	2.02	2.03	2.2	2.15	2.08	2.04	2.01
SEM	0.11	0.12	0.11	0.08	0.1	0.12	0.12	0.12

Table 603

Plasma motilin in patients with 'infective' diarrhoea.

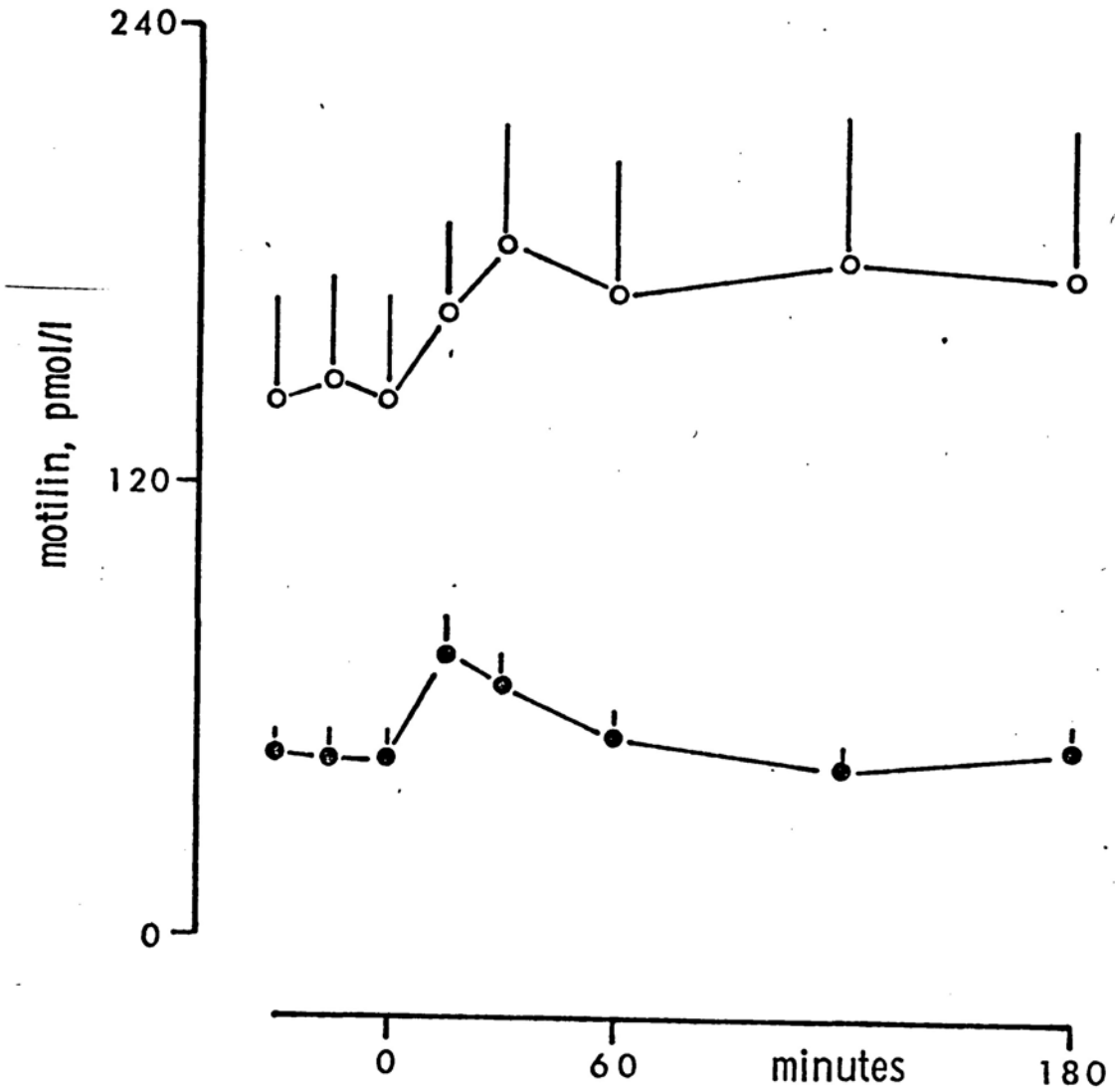


Figure 602

Plasma motilin (pmol/l) in patients with tropical malabsorption (open circles) and controls.

Sub	-30	-15	0	15	30	60	120	180
1	130	134	135	160	164	135	133	115
2	40	36	48	78	56	46	52	55
3	160	128	127	170	260	260	196	266
4	300	308	318	318	356	360	347	356
5	95	104	90	136	160	136	132	117
6	140	180	150	190	180	150	200	116
7	132	132	130	128	114	94	62	78
8	136	160	136	136	178	180	308	300
X	141.6	147.7	141.8	164.5	183.5	170	178.8	175.4
SEM	27.9	27.4	27.7	24.9	32.1	34.9	37.7	40.3
<u>log transformation</u> <u>of raw data</u>								
X	2.1	2.1	2.1	2.19	2.21	2.16		2.16
SEM	0.09	0.10	0.09	0.07	0.09	0.10	0.11	0.11

Table 604

Plasma motilin (pmol/l) in patients with Tropical Malabsorption.

Crohn's disease

Patients with Crohn's disease had elevated basal levels ($163 \pm 35 \text{ pmol/l}$ $p < 0.001$) when compared to the controls (Fig 603 and Table 605). The peak postprandial level occurred at 15 min after ingestion ($221 \pm 45 \text{ pmol/l}$) and this was significantly higher ($p < 0.001$) than the corresponding level in the controls. The incremental 15 min motilin rise in the patients with Crohn's disease ($58 \pm 15 \text{ pmol/l}$) was also significantly higher ($p < 0.01$) than the rise in the control group.

Ulcerative Colitis

The plasma motilin levels (both before and after the test meal) in the patients with ulcerative colitis were significantly higher than the control values ($p < 0.001$ throughout), (Fig 604 and Table 606). The incremental motilin rise at 30 min in the ulcerative colitis group although higher ($42 \pm 9.5 \text{ pmol/l}$) than the corresponding control value ($18 \pm 4.5 \text{ pmol/l}$) it was not statistically different.

Diverticular disease

The plasma motilin levels in the patients with diverticular disease are shown in Fig 605 and Table 607. Although the mean motilin levels were substantially higher than the control values, however statistical significance was not achieved owing to the considerable overlap between the motilin values in the two groups.

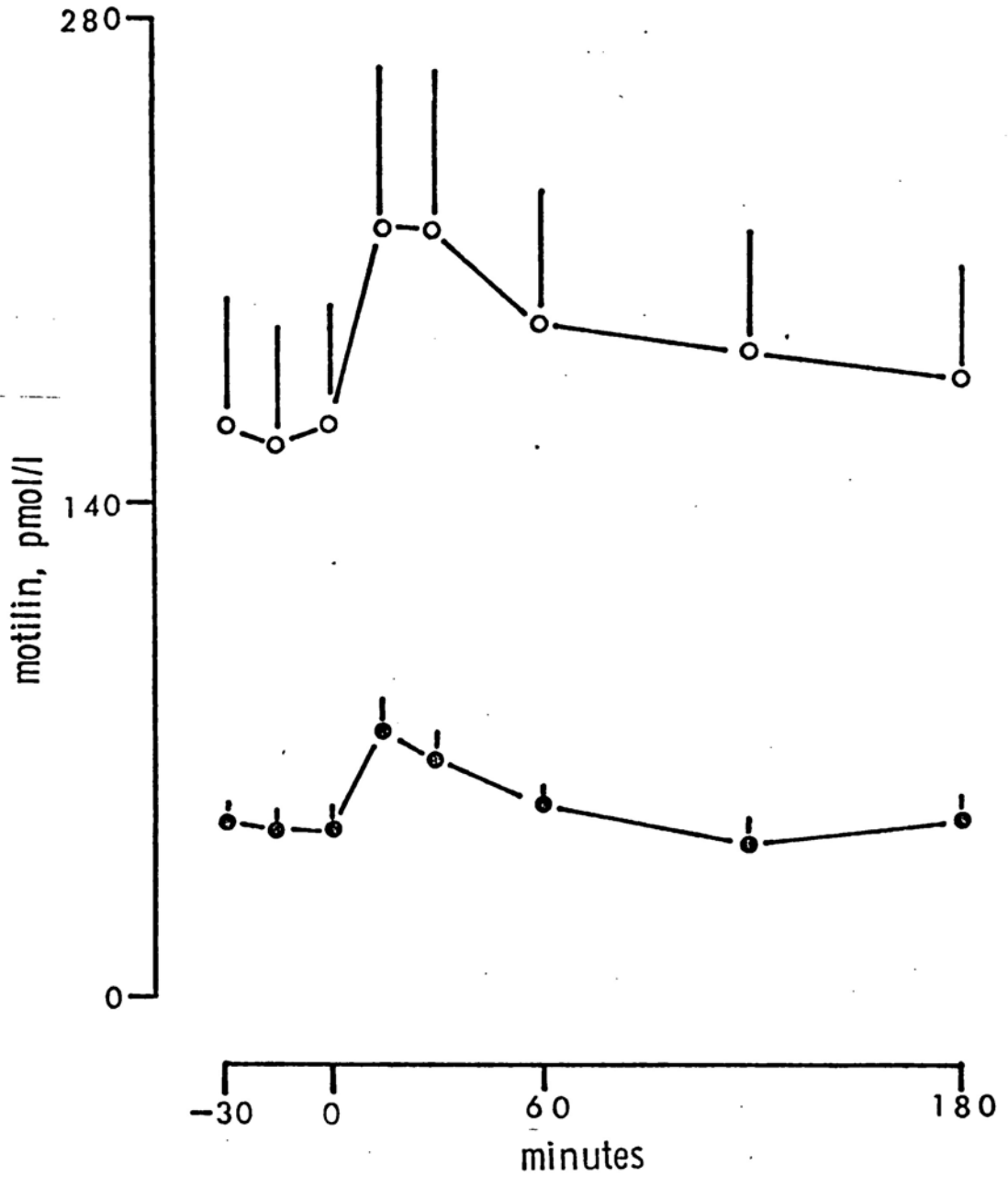


Figure 603

Plasma motilin (pmol/l) in patients with Crohn's disease (open circles) and controls.

Sub	-30	-15	0	15	30	60	120	180
1	246	226	216	388	364	264	216	178
2	92	104	130	180	180	122	124	120
3	294	240	300	374	382	336	316	300
4	16	20	16	60	76	68	98	56
5	28	20	26	60	44	54	66	112
6	166	180	194	192	172	184	128	144
7	98	106	102	144	112	104	102	90
8	10	8	10	56	22	20	20	40
9	202	220	194	180	188	224	254	258
10	500	500	500	660	660	554	460	488
11	100	104	128	166	154	144	126	148
12	188	138	130	232	256	262	166	120
13	112	126	152	166	164	146	136	142
14	224	196	184	230	284	280	364	272
X	162.6	156.3	163	220.6	218.4	191.3	184	176.3
SEM	36.3	34.8	35.1	44.8	46.2	38.6	34.4	32.8
<u>log transformation</u> <u>of raw data</u>								
X	2.02	2.01	2.04	2.24	2.20	2.18	2.16	2.16
SEM	0.14	0.14	0.14	0.09	0.11	0.10	0.10	0.08

Table 605

Plasma motilin (pmol/l) in patients with Crohn's disease.

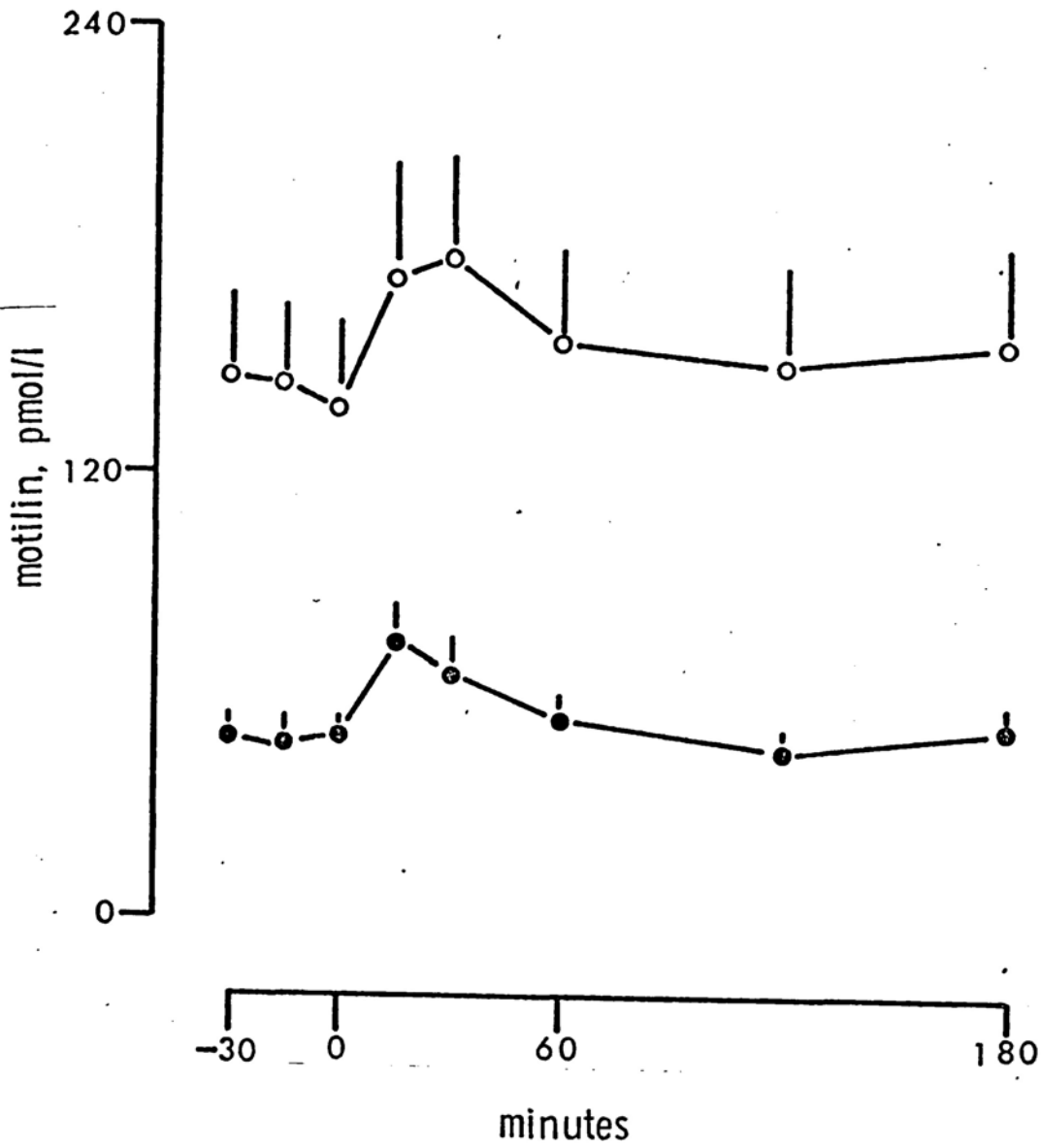


Figure 604

Plasma motilin (pmol/l) in patients with ulcerative colitis (open circles) and controls.

Sub	-30	-15	0	15	30	60	120	180
1	70	74	56	84	52	32	22	44
2	84	108	76	88	100	96	84	76
3	90	136	128	138	104	86	60	50
4	52	40	44	84	122	96	156	120
5	460	472	460	592	528	480	592	496
6	190	196	176	246	178	108	92	132
7	76	50	40	66	86	60	48	40
8	142	100	98	116	106	80	68	92
9	112	72	66	72	116	98	110	88
10	196	180	164	254	248	218	200	228
11	150	136	140	136	192	172	200	236
12	120	124	144	232	224	128	140	152
13	196	208	202	236	236	226	180	182
14	172	160	144	236	224	230	216	188
15	360	320	324	456	416	352	306	384
16	110	110	110	128	92	76	54	92
17	92	104	86	94	112	112	106	86
18	344	380	424	432	480	478	402	442
19	14	16	14	33	112	112	96	96
20	70	50	44	56	66	64	60	54
21	168	178	166	160	118	94	78	96
22	4	4	4	15	48	25	24	16
23	100	134	84	128	88	66	66	46
24	136	108	88	82	244	224	220	260
X	146.2	144.2	136.8	173.5	178.8	154.7	149.2	154
SEM	22.6	23.3	24.4	29.9	27.3	26.2	27.4	27
<u>log transformation</u>								
<u>of raw data</u>								
X	2.02	2.01	1.97	2.11	2.16	2.07	2.04	2.05
SEM	0.09	0.09	0.09	0.08	0.06	0.07	0.07	0.08

Table 606

Plasma motilin (pmol/l) in patients with ulcerative colitis.

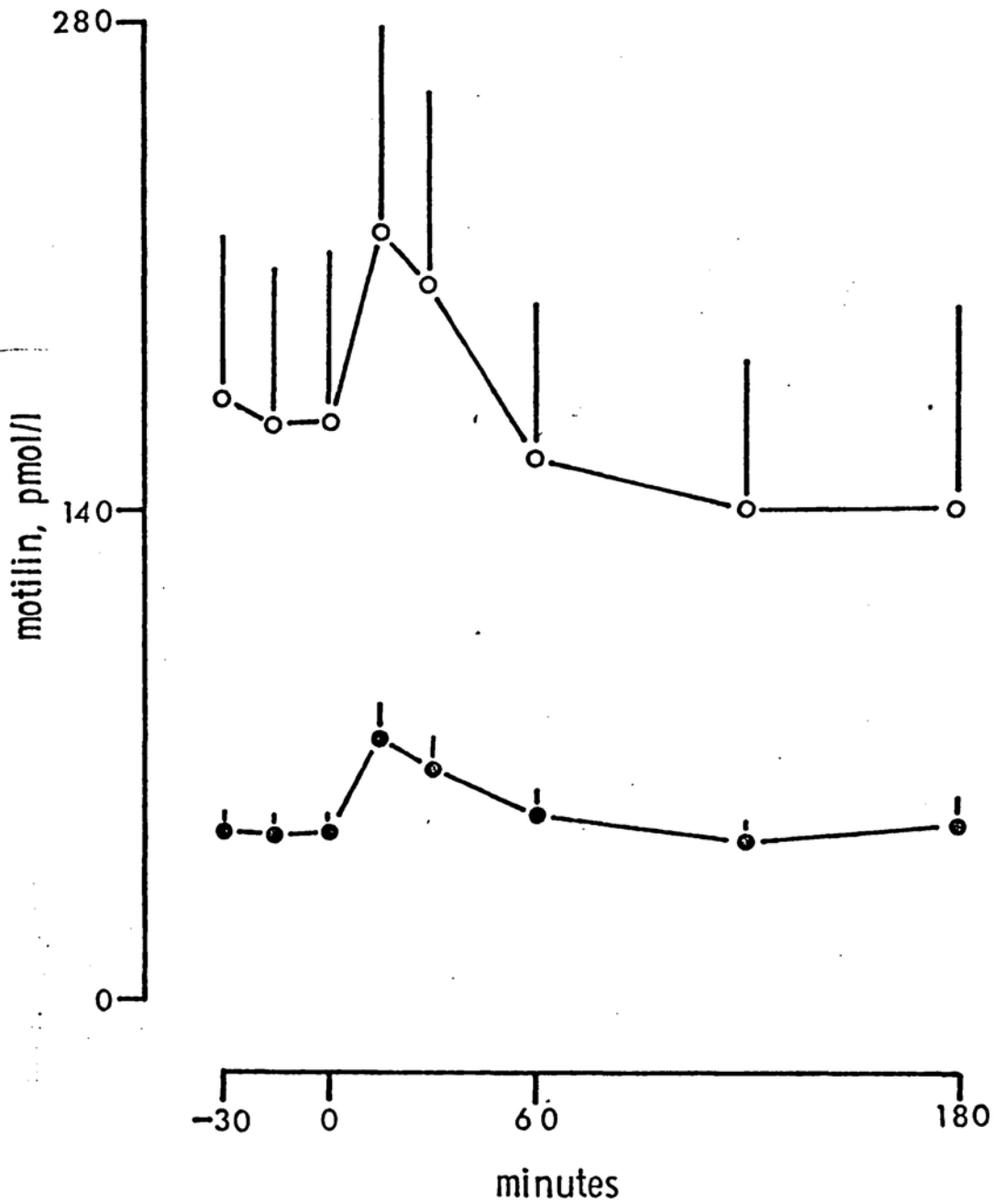


Figure 605

Plasma motilin (pmol/l) in patients with diverticular disease (open circles) and controls.

Sub	-30	-15	0	15	30	60	120	180
1	192	172	188	234	188	168	146	140
2	140	148	128	144	176	102	102	52
3	24	16	16	16	8	8	6	4
4	418	414	418	560	506	352	256	216
5	164	134	170	216	188	98	98	96
6	180	170	166	290	280	312	240	280
7	460	460	470	520	564	454	506	572
8	344	320	304	404	312	196	202	120
9	16	10	4	10	17	4	6	6
10	52	42	56	170	168	118	92	150
11	12	12	12	16	12	12	4	20
12	70	64	44	56	30	26	18	24
X	172.6	163.5	164.7	219.7	204.1	154.2	139.7	140
SEM	47.4	47.2	47.8	58	56.1	44.9	44.2	48.7
<u>log transformation</u> <u>of raw data</u>								
X	2.0	1.94	1.91	2.07	2.03	1.86	1.78	1.81
SEM	0.17	0.18	0.20	0.19	0.20	0.21	0.22	0.20

Table 607

Plasma motilin (pmol/l) in patients with diverticular disease.

Irritable Bowel Syndrome

The patients with irritable bowel syndrome and diarrhoea had only slightly elevated basal and postprandial motilin levels (Fig 606 and Table 608). These however did not reach statistical significance when compared with controls. The plasma motilin levels in the patients with constipation are shown in (Fig 607 and Table 609). The basal level of ($62 \pm 8.5 \text{ pmol/l}$) was not significantly different from the corresponding level in the controls ($48.5 \pm 6.5 \text{ pmol/l}$) or in the diarrhoea group ($67 \pm 14 \text{ pmol/l}$). The incremental motilin rise in the constipation group was however significantly lower than that seen in the patients with diarrhoea ($9 \pm 6.5 \text{ pmol/l}$ against $38.5 \pm 6.5 \text{ pmol/l}$, $p < 0.01$). Motilin levels did not change significantly following the meal in the constipation group.

Short Bowel Syndrome

Basal plasma motilin, in the patients with partial ileal resection, was ($190 \pm 44.5 \text{ pmol/l}$) rising postprandially to a peak at 30 min (■) ($279 \pm 61.5 \text{ pmol/l}$) ($p < 0.001$). Both, the basal and the incremental motilin rise were significantly higher than the controls ($p < 0.001$) (Fig 608 and Table 610).

Plasma motilin levels in the patients who had undergone partial colectomy were not significantly different from control levels (Fig 609 and Table 611).

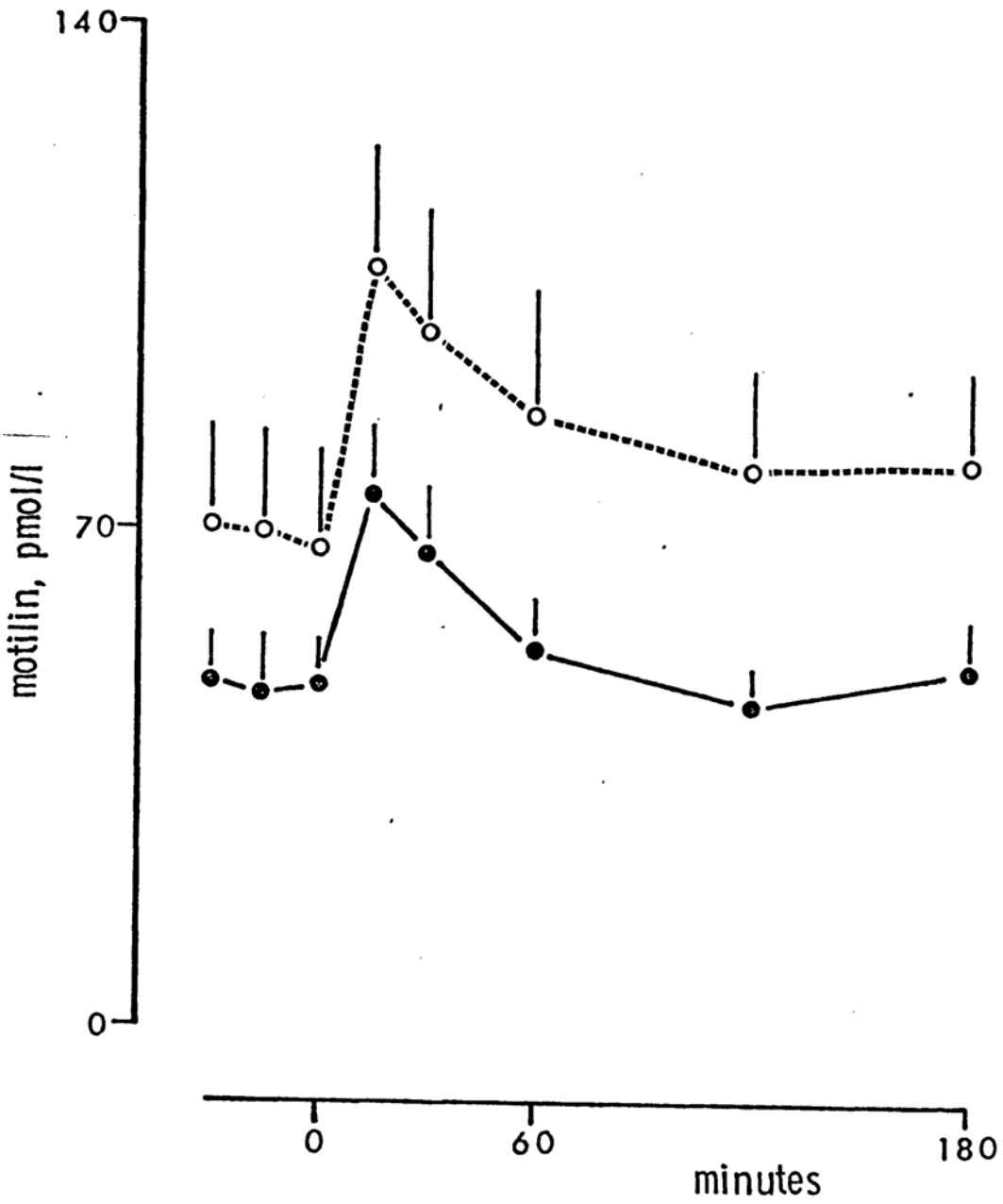


Figure 606

Plasma motilin (pmol/l) in patients with IB3 and diarrhoea (open circles) and controls.

Sub	-30	-15	0	15	30	60	120	180
1	97	90	76	154	204	135	174	150
2	87	76	96	165	130	80	94	135
3	20	16	16	30	36	28	27	29
4	147	126	151	234	234	228	144	144
5	160	168	167	170	170	218	190	165
6	26	22	26	35	39	28	20	17
7	55	42	39	91	62	58	62	58
8	21	24	25	77	41	21	17	22
9	10	16	12	25	18	14	11	20
10	46	40	32	80	78	67	54	56
11	32	33	30	35	34	14	28	34
12	78	70	58	93	62	62	49	43
13	185	213	210	240	212	136	115	168
14	64	62	64	102	113	78	98	86
15	30	36	34	73	60	62	52	65
16	184	194	152	238	188	254	180	156
17	6	6	7	8	6	4	15	8
18	27	28	24	52	58	46	42	43
19	66	52	59	110	101	92	115	102
X	70.6	69.2	67.3	105.9	97.2	85.5	78.3	79
SEM	13.8	14.7	14.1	17.5	16.9	17.8	14.1	13.4
<u>log transformation</u> <u>of raw data</u>								
X	1.68	1.67	1.66	1.89	1.87	1.74	1.75	1.76
SEM	0.10	0.10	0.10	0.09	0.10	0.11	0.09	0.09

Table 608

Plasma motilin (pmol/l) in patients with IBS and diarrhoea.

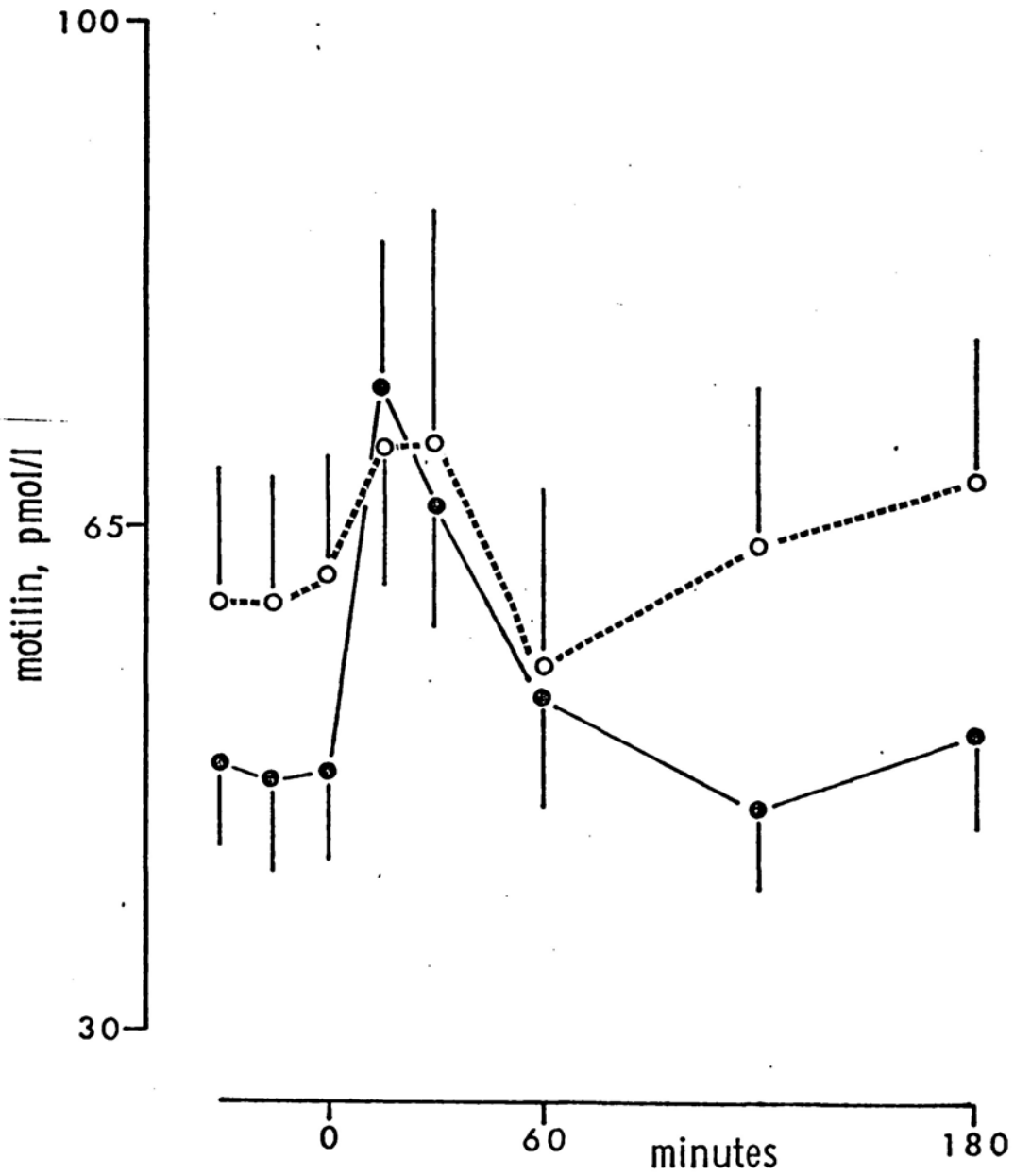


Figure 607

Plasma motilin (pmol/l) in patients with IBS and constipation (open circles) and controls.

Sub	-30	-15	0	15	30	60	120	180
1	77	77	94	77	85	39	38	30
2	65	69	60	46	70	44	90	99
3	42	40	40	58	37	42	44	60
4	18	18	26	29	14	8	11	24
5	46	48	52	35	32	18	28	22
6	130	124	115	125	124	121	100	106
7	86	84	86	104	78	83	89	98
8	38	34	40	68	40	37	42	63
9	51	48	57	110	167	127	125	100
10	40	49	44	56	86	48	64	68
11	60	65	64	68	70	44	68	78
X	59.4	59.6	61.6	70.5	73	55.5	63.5	68
SEM	9.6	9.1	8.4	9.8	13.9	12.2	11	10
<u>log transformation</u>								
<u>of raw data</u>								
X	1.72	1.73	1.75	1.81	1.78	1.64	1.72	1.77
SEM	0.07	0.07	0.06	0.06	0.10	0.11	0.10	0.08

Table 609

Plasma motilin (pmol/l) in patients with IBS and constipation.

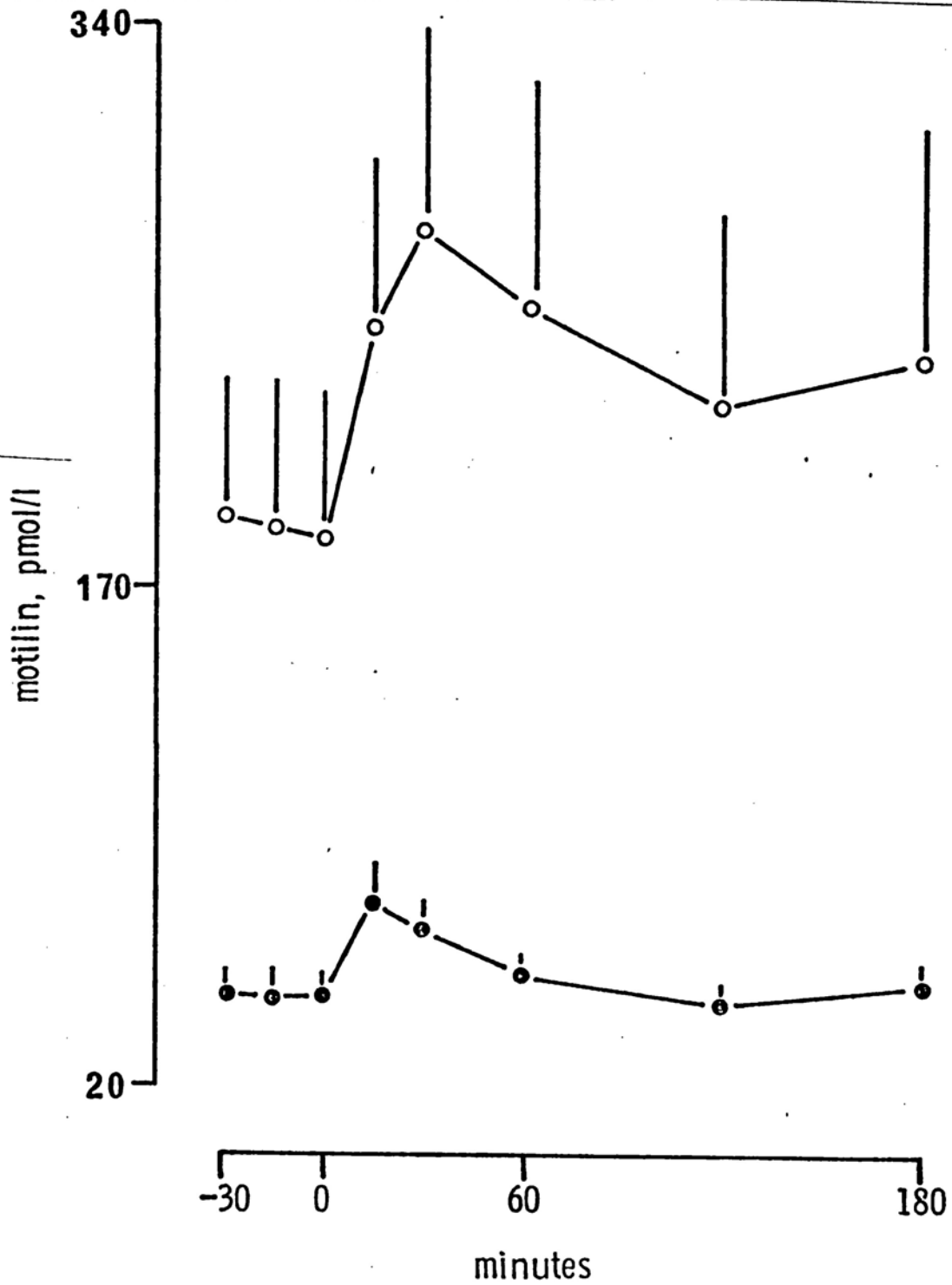


Figure 608

Plasma motilin (pmol/l) in patients undergone partial ileal resection (open circles) and controls.

Sub	-30	-15	0	15	30	60	120	180
1	232	210	228	222	169	116	67	92
2	228	213	206	260	350	258	238	214
3	266	242	225	258	270	245	196	274
4	24	19	22	52	55	43	55	54
5	220	208	228	300	264	248	228	168
6	348	384	360	360	360	320	336	336
7	532	600	528	720	728	872	688	688
8	600	632	712	816	1040	1040	920	1200
9	160	116	168	380	476	464	400	344
10	60	54	44	38	141	101	81	77
11	15	17	27	81	44	50	40	48
12	174	180	114	157	164	152	203	203
13	41	24	12	38	96	35	26	31
14	28	16	22	126	70	54	86	58
15	238	167	123	145	234	149	112	144
16	52	60	55	122	253	140	108	103
17	106	86	92	125	123	132	97	98
18	140	184	170	275	178	200	176	196
X	192.4	189.6	185.3	248.6	278.6	256	225.4	240.4
SEM	40.6	44.6	45.4	52.6	61.6	67.6	57.5	69.5
<u>log transformation</u>								
<u>of raw data</u>								
X	2.09	2.05	2.04	2.25	2.3	2.22	2.17	2.18
SEM	0.11	0.12	0.12	0.09	0.09	0.10	0.10	0.10

Table 610

Plasma motilin (pmol/l) in patients undergone partial ileal resection.

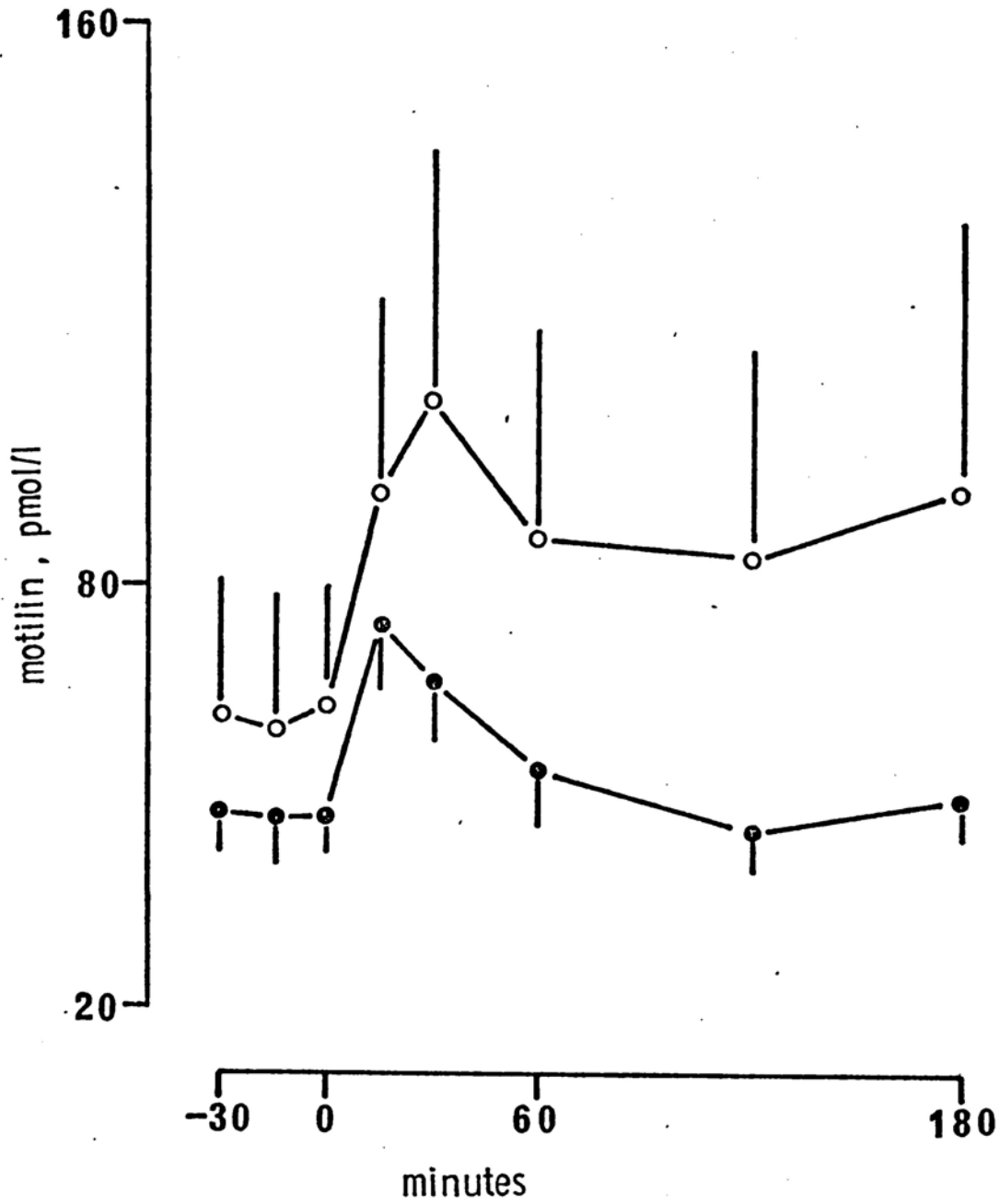


Figure 609

Plasma motilin (pmol/l) in patients undergone partial colectomy (open circles) and controls.

Sub	-30	-15	0	15	30	60	120	180
1	85	90	95	231	258	240	240	260
2	21	10	34	56	48	18	32	33
3	62	53	81	65	76	65	63	48
4	6	13	6	15	19	10	13	17
5	178	184	158	169	276	208	208	296
6	50	46	45	41	28	44	25	36
7	3	8	15	11	8	2	5	7
8	38	34	57	84	102	93	70	50
9	110	92	100	168	150	103	98	102
X	61.4	58.9	62.7	93.3	107	87	83.8	94.3
SEM	19.9	20	17	27.7	35.7	30.3	30.1	38.1
<u>log transformation</u> <u>of raw data</u>								
X	1.54	1.57	1.66	1.79	1.80	1.63	1.67	1.71
SEM	0.21	0.17	0.16	0.16	0.19	0.29	0.19	0.19

Table 611

Plasma motilin (pmol/l) in patients undergone partial colectomy

DISCUSSION

This chapter describes the work that was undertaken to analyse plasma motilin concentrations in gastrointestinal disorders. In total, 116 patients were studied, suffering from diarrhoea (of different aetiology). The results presented here show that $60 \pm 9\%$ of all 116 patients with diarrhoea have plasma motilin levels higher than the 95% centile of the control group (ie 100pmol/l). Dividing the patients into their respective disease groups it became apparent that a higher proportion of elevated motilin concentrations occurred in patients with Crohn's disease (79%), small intestinal resection (78%) and Tropical malabsorption (75%). The lowest proportion of elevated motilin concentrations occurred in the Irritable Bowel Syndrome (and diarrhoea) (21%) large intestinal resection (33%), ulcerative colitis (50%) diverticular disease (58%) and infective diarrhoea (58%). These results suggest that motilin may have an, as yet unknown, relationship with pathogenesis of some types of diarrhoea.

The cause of diarrhoea in these disease groups is poorly understood. Pathogenic mechanisms are thought to include the following (145):-

1. Excessive Intraluminal Volume. In these conditions excessive fluid accumulations are chiefly due to one of the following:-

- a. Decreased absorption of water and solute (osmotic diarrhoea), including that resulting from diseases such as Tropical Malabsorption and coeliac disease.

b. Increased secretion into the lumen, of which the classic model is cholera. Recent evidence makes it clear that the same mechanism is also active in infection with enteropathogenic E. Coli and other gram-negative organisms. Abnormal secretion into the colonic lumen is characteristic of diarrhoea caused by ileal resection, resulting from the action of excess bile acids on colonic mucosa and also of increased intestinal distension which results in an exaggerated propulsive motility.

2. Exaggerated Propulsive motility. This is often an independent and important variable. The most common example is the irritable bowel syndrome.

In many bowel diseases, more than one of these mechanism are thought to be operative at one time and these may vary in individual patients.

The complexity and the unknown involvement of gastrointestinal motor activity in the pathogenesis of diarrhoea makes any interpretation of the motilin results, almost impossible. A further complication in the interpretation of these results, is the presence of a second 'molecular form' of motilin in plasma, the nature of which is not known. Its presence in the plasma of patients in the above diseases and its relative contribution towards the elevated motilin concentration remains to be established.

The finding of an absent postprandial motilin release in the patients with Irritable Bowel Syndrome and constipation was of considerable interest. These patients have been reported to lack a gastrocolic response following a meal (146) and this appears to be in accord with the putative role of

motilin in the control of colonic motility (27,147).

Research into the 'pathophysiological' involvement of motilin has been very limited. It has been reported that plasma motilin levels are not different from normal values in duodenal ulceration (148), anorexia nervosa (149) and Chagas's disease (150). In addition no abnormalities of motilin release were found in patients with the dumping syndrome (151) and thyrotoxicosis, (149) both diseases characterised by abnormally rapid gastrointestinal motility. Elevated motilin levels have been reported in patients with chronic pancreatitis, (153) who are thought to have abnormally rapid gastric emptying (153). Suppressed motilin concentrations were found in patients with postoperative ileus (154), with an elevation of levels occurring postoperatively and this elevation correlating with the return of normal colonic motility (154).

These preliminary studies suggest that motilin could be implicated in the pathogenesis of diarrhoea, via its actions on gastrointestinal motility but the exact mechanism is not understood. Future research employing the simultaneous study of gut motility and plasma motilin could prove very rewarding.

CHAPTER 7

CONCLUSIONS

Although the discovery of motilin is a relatively recent event considerable progress has been made in establishing motilin as a circulating gastrointestinal hormone. Motilin is localised in distinct endocrine cells which are found in the duodenum and jejunum. Motilin circulates in plasma and levels are modified in response to various stimuli. Motilin has a wide spectrum of motor actions, some of which are seen at plasma concentrations similar to those seen after 'natural' stimuli. In addition, circulating motilin concentrations appear to be influenced by gastrointestinal diseases. There are however, several questions still unresolved.

The radiimmunoassay of motilin has proven to be relatively simple compared with that of other gastrointestinal hormones. Motilin antibodies were obtained in the majority of the rabbits immunised, and some of these proved to be of sufficiently high affinity for use in radioimmunoassay. Also, as the antibody used was not significantly affected by 'non-specific interference factors' the estimation of plasma motilin was straightforward. Preparation of the iodinated motilin presented no special difficulty, with the label achieving a high specific activity and stable for several months. The assay proved to be reliable, exhibiting a small intra - and inter - assay variation. The plasma concentrations of motilin are of a higher magnitude than most of the gastrointestinal hormones and therefore assay sensitivity did not constitute a problem.

Motilin was released into circulation following ingestion of a mixed meal. The postprandial release of motilin appeared to be complex. Fat, administered orally or via the intravenous route, and gastric distension appeared to stimulate the release of motilin, whereas oral or intravenous glucose and aminoacids caused a suppression. Also, other

gastrointestinal hormones tended to either stimulate (ie bombesin) or inhibit its release (ie PP, insulin and somatostatin). The effect of the nervous system was not fully investigated. Preliminary results suggested that the beta-adrenergic pathway was not involved (antral balloon distension experiments), and neither was the vagus (water load experiment). However the fasting motilin levels appeared to be under cholinergic control. Although it is clear that both a direct and indirect involment of food exists in the normal postprandial release of motilin, the exact nature of these mechanisms are still unresolved.

Regionally specific antibodies were used to characterise motilin immunologically in several species. It became apparent that motilin not only exhibited a species heterogeneity but also a molecular heterogeneity. Indeed, it was found that none of the antibodies (either C or N - terminally specific) crossreacted with intestinal extracts from various species, including rat, mouse, rabbit and frog. Motilin - like immunoreactivity from canine intestine was only measurable with C-terminally specific antibodies, whereas N-terminally specific antibodies showed minimal crossreaction, suggesting changes in the N-terminal sequence. Gel chromatography of human intestinal extracts revealed the presence of an additional immunoreactive motilin peak, which eluted earlier than the porcine motilin standard. The finding that C-terminal specific antibodies poorly crossreacted with this apparently high molecular size peak suggested that this substance may contain the sequence of 'small' motilin with an elongation at the C-terminal end. Examination of different regions of human intestine showed similar relative concentrations of the two immunoreactive motilin peaks along the intestine. The existence of an apparently higher molecular size substance, eluting in the same position as the tissue form, was also shown in human plasma. Analysis of plasmas

following various stimuli showed that both motilin peaks changed in parallel. However, infusion of porcine motilin resulted in a suppression of the earlier immunoreactive motilin peak whereas the later peak showed the expected rise. These findings suggest that the two immunoreactive motilin peaks may be biosynthetically related. Future purification of the larger form will allow a definitive study of its sequence and pharmacology.

When infused into man, at concentrations which mimic physiological levels, motilin can stimulate the gastric emptying of a solid meal marker and liquid glucose. The acceleration of the gastric emptying of glucose by motilin is thought to be responsible for the enhanced post-glucose response of plasma insulin and glucose. Indeed, this observation and the fact that intravenous glucose inhibits the release of motilin has led to the speculation of a feedback control mechanism, with glucose controlling its own absorption, via its action on motilin and the resultant gastric emptying. Motilin infusion studies in both dog and man have suggested a role of motilin in the control of interdigestive intestinal motility. Motilin has been shown to initiate the migrating motor-complexes which sweep down the intestine in the interprandial period and are thought to be responsible for keeping the intestine free of debris and undigested food. Motilin has also been shown to stimulate the large bowel and the gallbladder. The results to date suggest that motilin may have a role in the control of gastrointestinal motility, although more work on the exact mechanisms and dose-response effects are still required.

It is of interest that motilin release is exaggerated in conditions in which the gastrointestinal propulsive activity is often thought to be exaggerated, such as in patients with diarrhoea. However the exact involvement of gut motility in these conditions is poorly understood. Study of gut motility

in these patients with simultaneous motilin measurements may help towards our understanding of the pathophysiology of motilin.

Future studies will no doubt unravel the complex release mechanisms of motilin and perhaps also bring to light as yet unknown physiological actions and involvement in human disease.

APPENDIX 1

RECIPES FOR BUFFERS

1. Acetate buffer (0.05M) pH 5.0

4.1g sodium acetate/ litre distilled water

2. Freeze drying solution

10g lactose + 5g Bovine Serum Albumin (BSA) + 420mg citric acid + 200mg L-Cysteine hydrochloride + 160,000 KIU Aprotinin (Trasylol) + 200ml of 0.1M formic acid

3. Phosphate buffer (0.02M) pH 7.4

- a. 2.72g/100ml Potassium dihydrogen orthophosphate
- b. 3.56g/100ml di-Sodium hydrogen orthophosphate dihydrate

Mix 19.7ml of (a) with 80.3ml of (b)

4. Phosphate EDTA buffer (0.06M) pH 7.4

To make 5 litres :

42.88g di-Sodium hydrogen orthophosphate dihydrate
8.05g Potassium dihydrogen orthophosphate
2.5g Sodium azide
16.8g Ethylenediaminetetra-acetic acid, disodium salt

Pre-boil 5 litres of distilled water. Dissolve 12.5g of gelatine in this just boiled water by leaving to stir for about 15 min. When cool dissolve the constituents

5. Sorensens buffer (0.06M) pH 7.0

To make 5 litres :

19.7g Potassium dihydrogen orthophosphate
27.8g di-Sodium hydrogen orthophosphate dihydrate
2.5g Sodium azide

Pre-boil 5 litres of water. Dissolve 12.5g of gelatine in this just boiled water by leaving to stir for about 15 min. When cool dissolve the constituents

6. Veronal buffer (0.05M) pH 8.0

To make 5 litres :

51.5g Sodium barbitone
20 ml 5M hydrochloric acid
2.5g Sodium azide

Pre-boil 5 litres of water. Dissolve 12.5g of gelatine in this just boiled water by leaving to stir for about 15 min. When cool dissolve the sodium barbitone completely before adding the acid. Allow the acid to disperse well and then add the azide.

APPENDIX 2

CHEMICALS AND EQUIPMENT

1. Chemicals

- Acetic acid, glacial, BDH Chemicals Ltd, Poole, England
- Ammonium Hydrogen carbonate, Hopkin & Williams, Essex
England
- Ammonium Sulphate, BDH Chemicals Ltd, Poole, England
- Arlacel A, Sigma, St. Louis, USA
- Barbitone sodium, BDH Chemicals Ltd, Poole, England
- Bovine Serum Albumin (crystallised), Armour Pharmaceutical,
England
- Charcoal (Norit GSX), Hopkin & Williams, Essex, England
- Chloramine T, BDH Chemicals Ltd, Poole, England
- Citric acid, BDH Chemicals Ltd, Poole, England
- Cyanogen Bromide - activated Sepharose 4B, Pharmacia,
Uppsala, Sweden
- L-Cysteine hydrochloride, BDH Chemicals Ltd, Poole,
England
- Cytochrome C, type VI (from horse heart), Sigma,
St. Louis, USA
- Dextran, Clinical grade (M.W.=80,000), Sigma, St. Louis, USA
- Dextran Blue, Sigma, St. Louis, USA
- 1-ethyl-3-(3-Dimethylaminopropyl)- carbodiimide, Sigma,
St. Louis, USA
- Ethylenediaminetetra-acetic acid, disodium salt,
BDH Chemicals Ltd, Poole, England
- Formic acid, BDH Chemicals Ltd, Poole, England
- Gelatine, powder, BDH Chemicals Ltd, Poole, England
- D-glucose, BDH Chemicals Ltd, Poole, England
- Hexadecane, puriss, Koch-Light, Colnbrook, England
- Hydrochloric acid, BDH Chemicals Ltd, Poole, England
- Hydrogen Peroxide, Hopkin & Williams, Essex, England
- Lactoperoxidase (from milk), Sigma, St. Louis, USA
- Lactose, BDH Chemicals Ltd, Poole, England
- Potassium dihydrogen orthophosphate, BDH Chemicals Ltd,
Poole, England

Potassium hydroxide, BDH Chemicals Ltd, Poole, England
Potassium iodide, BDH Chemicals Ltd, Poole, England
Sephadex G-50 superfine, Pharmacia, Uppsala, Sweden
Sodium acetate (anhydrous), BDH Chemicals Ltd,
Poole, England
Sodium azide, BDH Chemicals Ltd, Poole, England
Sodium chloride, Hopkin & Williams, Essex, England
di-Sodium hydrogen orthophosphate dihydrate,
BDH Chemicals Ltd, Poole, England
Sodium hydroxide, BDH Chemicals Ltd, Poole, England
Sodium Iodide (125), Amersham, England
Sodium metabisulphite, BDH Chemicals Ltd, Poole, England
Tris (hydroxymethyl methylamine) BDH Chemicals Ltd,
Poole, England
Urea, BDH Chemicals Ltd, Poole, England.

2. Equipment

Balances - Cahn electrobalance, California, USA
Oertling balance, London, England
Centrifuges - Small centrifuge, EBA 35, A.R. Horwell,
London, England
Coolspin, MSE, Fisons, England
Chromatography - Fraction collector, type 270,
Chemlab, Essex, England
Minipump, Model 1, Schuco Scientific Ltd,
London, England
Pharmacia columns, K9/60, Pharmacia, Uppsala,
Sweden
Polypropylene drums, Chemlab, Essex, England
Dispensers - Graduated syringe (reference 1),
A.R. Horwell Ltd, London, England
Holders/teats for volupettes, Medikent,
England
Repeater hamilton syringe, Hamilton, Bonaduz,
Switzerland
Single hamilton syringe, Hamilton, Bonaduz,
Switzerland
Volupettes, Medikent, England

Gamma-counter - NE 1600, Nuclear Enterprises,
Edinburgh, Scotland

Homogeniser - Ultra-turrax, Scientific instruments,
London, England

Lyophiliser - Edward high vacuum, Edwards, Sussex, England

pH meter - Corning pH meter, model 5, Corning, England

Refrigeration - ESTA 601 freezer, Bejam, London, England
Osbourne bottle cooling trays, LEC refrigerator
service, London, England

Tubes - GW3, polystyrene tubes, Seward Lab, London,
England

GW4, polystyrene tubes, Seward Lab., London,
England

Freeze drying glass vials, Anchor Glass, London,
England

LH/10 Lithium Heparin containers, Seward Lab.,
London, England

LP3, polystyrene tubes, Luckham Ltd., Sussex,
England

Universal containers, Sterilin, England

Z/5 plastic blood containers, Seward Lab, London,
England

REFERENCES

1. Berthold, A.A. : Transplantation der Hoden Arch. Anat. Physiol. Wiss. Med 16 : 42, 1849
2. Forbes, T.R. : A.A. Berthold and the first endocrine experiment : some speculation as to its origin. Bull. Hist. Med 23 : 263, 1949
3. Bernard, C : On the mechanism of formation of sugar in the liver : Compt. Rend. Acad. Sci. 41 : 461, 1855 (as translated in great experiments in Biology, edited by M.L. Gabril and S. Fogel. Englewood Cliffs, N.J. : Prentice-Hall, 1955).
4. Mering, J, von and Minkowski, O. : Diabetes mellitus nach Pankreasextirpation. Naunyn - Schmiedeberg's. Arch. Exptl. Pathol. Pharmacol. 26 : 373, 1890
5. Bayliss, W.M. and Starling, E.H. : The mechanism of pancreatic secretion. J Physiol (London). 28 : 325, 1902
6. Starling, E.H. : The Croonian Lecture on the Chemical correlation of the function of the body I. Lancet, ii : 339, 1905
7. Mutt, V.: Preparation of highly purified secretin. Arkiv. Kemi 15 : 69, 1959
8. Shay, H. and Gershon-Cohen, J. : Experimental studies in gastric physiology in man : the mechanism of gastric evacuation after partial gastrectomy as demonstrated roentgenologically. AM J. Dig Dis 2 : 608, 1935
9. Brown, JC, Johnson, LP, and Magee, DF : Effect of duodenal alkalisation on gastric motility. Gastroenterology 50 : 333, 1966
10. Brown, JC : The presence of a gastric motor stimulating property in duodenal extracts. Gastroenterology 52 : 225, 1967
11. Brown, JC and Parkes, C.O. : Effect on fundic pouch motor activity of stimulatory and inhibitory fractions separated from Pancreozymin. Gastroenterology 53 : 73, 1967
12. Brown, JC and Peterson, RA : A multiparameter study on the action of preparations containing cholecystokinin - pancreozymin. Scand J. Gastroent 5 : 537, 1970
13. Mutt, V : Chromatography of secretin on carboxymethyl cellulose. Acta Chem. Scand 13 : 1247, 1959.

14. Brown, JC, Mutt, V. and Dryburgh, JR : The further purification of motilin, a gastric motor activity - stimulating polypeptide from the mucosa of the small intestine of hogs. *Can. J. Physiol. Pharmacol.* 49 : 399, 1971
15. Brown, JC, Cook, M.A., and Dryburgh, JR : Motilin, a gastric motor activity - stimulating polypeptide : final purification, amino acid composition, and C-terminal residues. *Gastroenterology*, 62 : 401, 1972
16. Brown, J.C., Cook, M.A., and Dryburgh, J.R. (1973) : Motilin, a gastric motor activity stimulating polypeptide : the complete amino acid sequence. *Can. J. Biochem* 51 : 533, 1973
17. Wunsch, E., Brown, J.C., Deimer, K.H., Drees, F., Jaeger, E., Musiol, J., Scharf, R., Stocker, H., Thamm, P., and Wendlberger, G. : The total synthesis of norleucine-13-motilin. *Z. Naturforschung*, 28 : 235, 1973
18. Yajima, H., Kai, Y., and Kawatani, H. : Synthesis of the docosa-peptide corresponding to the entire amino acid sequence of porcine motilin. *J. Chem. Soc. Chem. Commun.* 150, 1975
19. Shimizu, F., Imagawa, K., Mihara, S., and Yanaihara, N : Synthesis of porcine motilin by fragment condensation using three protected peptide fragments. *Bull. Chem. Sci. Japan.* 49 : 3594, 1976
20. Segawa, T., Nakano, M., Kai, Y., Kawatani, H., and Yajima, H. : Effect of synthetic motilin and related polypeptides on contraction of gastrointestinal smooth muscle. *J. Pharm. Pharmacol.*, 28 : 650, 1976
21. Yajima, H., Kai, Y., Ogawa, H., Kubota, M., Mori, Y., and Koyama, K. : Structure - activity relationships of gastrointestinal hormones : motilin, GLP and (27-TYR) CCK-PZ. *Gastroenterology*, 72 : 793, 1977
22. Bloom, S.R., Mitznegg, P., and Bryant, M.G. : Measurement of human plasma motilin. *Scand. J. Gastroent.* (suppl. 39), 11 : 47, 1976
23. Pearse, A.G.E., Polak, J.M., Bloom, S.R., Adams, C., Dryburgh, J.R., and Brown, J.C. : Enterochromaffin cells of the small intestine as the source of motilin. *Virchows Arch.* (Cell Pathol) 16 : 111, 1974
24. Helmstaeder, V., Kreppein, W., Domschke, W., Mitznegg, P Yanaihara, N., Wunsch, E. and Forssman, W.G. : Immunohistochemical localisation of motilin in endocrine non-enterochromaffin cells of the small intestine of human and monkey. *Gastroenterology*, 76 : 897, 1979.

25. Christofides, N.D., Bryant, M.G., Ghatei, M.A., Kishimoto, S., Buchan, A.M.J., Polak, J.M., and Bloom, S.R. : Molecular forms of motilin in the mammalian and human gut and human plasma. *Gastroenterology*, 80 : 292, 1981
26. Strunz, U., Domschke, W., Domschke, S., Mitznegg, P., Wunsch, E., Jaeger, E., and Demling, L. : Gastroduodenal motor response to natural motilin and synthetic position 13-substituted motilin analogues : a comparative in vitro study. *Scand. J. Gastroent.* (Suppl. 39) 11 : 199, 1976
27. Strunz, U., Domschke, W., Mitznegg, P., Domschke, S., Schubert, E., Wunsch, E., Jaeger, E., and Demling, L. : Analysis of the motor effects of 13-norleucine motilin on the rabbit, guinea-pig, rat and human alimentary tract in vitro. *Gastroenterology*, 68 : 1485, 1975
28. Domschke, W., Strunz, U., Mitznegg, P., Domschke, S., Springel, W., Ruppel, H., Wunsch, E., and Demling, L. : Pharmacology of motilin. In : *Gut Hormones*, edited by S.R. Bloom, Churchill Livingstone Press, p335.
29. Schubert, E., Mitznegg, P., Strunz, U., Domschke, W., Domschke, S., Wunsch, E., Jaeger, E., Demling, L., and Hein, F. : Influence of the hormone analogue 13-Nle-motilin and 1-methyl-3-isobutylxanthine on tone and cyclic 3',5'-AMP content of antral and duodenal muscles in the rabbit. *Life Sci.*, 16 : 263, 1975
30. Konturek, S.J., Dembinski, A., Krol, R., and Wunsch, E. : Effects of motilin on gastric and pancreatic secretion in dogs. *Scand. J. Gastroent* (Suppl 39), 11 : 57, 1976
31. Domschke, S., Domschke, W., Schmack, B., Tympner, F., Junge, O., Wunsch, E., Jaeger, E., and Demling, L. : Effects of 13-Nle-motilin on salivary, gastric, and pancreatic secretions in man. *Am. J. Dig. Dis*, 21 : 789, 1976
32. Mitznegg, P., Domschke, W., Domschke, S., Belohlavek, D., Sprugel, W., Strunz, U., Wunsch, E., Jaeger, E., and Demling, L. : Effects of in vivo administration of pentagastrin, secretin and 13-Nle-motilin on the in vitro incorporation of 14 C-leucine into protein of human gastric mucosa. *Scand. J. Gastroent.*, 11 : 657, 1976
33. Code, C.F., and Marlett, J.A. : The interdigestive myo-electric complex of the stomach and small bowel of dogs, *J. Physiol.* 246 : 289, 1975

34. Itoh, Z., Honda, R., Hiwatashi, K., Takeuchi, S., Aizawa, I., Takayanagi, R., and Couch, E.E. : Motilin-induced mechanical activity in the Canine Alimentary Tract. Scand. J. Gastroent., (Suppl 39) 11 : 93, 1976
35. Wingate, D.K., Ruppín, H., Green, W.E.R., Thompson, H.H., Domschke, W., Wunsch, E., Demling, L., and Ritchie, H.D. : Motilin-induced electrical activity in the canine gastrointestinal Tract. Scand. J. Gastroent. (Suppl. 39), 11 : 111, 1976
36. Itoh, Z., Takeuchi, S., Aizawa, I., Takayanagi, R., Mori, K., Taminato, T., Seino, Y., Imura, H., and Yanaihara N : Recent advances in motilin research : its physiological and clinical significance. In Advances, in Experimental Medicine and Biology, edited by M. Grossman, V., Speranza, N. Basso and E. Lezoché, 106, 241
37. Ruppín, H., Sturm, G., Westhoff, D., Domschke, S., Domschke, W., Wunsch, E., Demling, L. : Effect of 13-Nle-motilin on small intestinal transit time in healthy subjects. Scand. J. Gastroent (Suppl. 39) 11, 85, 1976
38. Ruppín, H., Domschke, S., Domschke, W., Wunsch, E., Jaeger, E., and Demling, L. : Effects of 13-Nle-motilin in man-inhibition of gastric evacuation and stimulation of pepsin secretion. Scand. J. Gastroent., 10 : 199, 1975
39. Debas, H.T., Yamagishi, T., and Dryburgh, J.R. : Motilin enhances gastric emptying of liquids in dogs. Gastroenterology, 73, 777, 1977
40. Nakazawa, S., Segawa, K., Hidano, H., Tsukamoto, Y., Aichi, M., and Ichikawa, T. : The effect of motilin on the gastrointestinal propulsion in rats. Gastroent. (Japonica), 13 : 50, 1978
41. Jennewein, H.M., Hummelt, H., Siewert, R., and Waldeck, F. : The motor-stimulating effect of natural motilin on the lower oesophageal sphincter, fundus, antrum, and duodenum in dogs. Digestion, 13 : 246, 1975
42. Jennewein, H.M., Bauer, R., Hummelt, H., Lepsin, G., Siewert, R., and Waldeck, F. : Motilin effects on gastrointestinal motility and lower oesophageal sphincter (LES) pressure in dogs. Scand. J. Gastroent (Suppl. 39), 11 : 63, 1976
43. Meissner, A.J., Bowes, K.L., Zwick, R. and Daniel, E.E. : Effect of motilin on the lower oesophageal sphincter. Gut, 17 : 923, 1976

44. Lux, G., Rosch, W., Domschke, S., Domschke, W., Wunsch, E., Jaeger, E. and Demling, L. : Intravenous ^{131}I -motilin increases the human lower oesophageal sphincter pressure. Scand. J. Gastroent. (Suppl. 39) 11 : 75, 1976
45. Shima, K., Shin, S., Tanaka, A., Hashimura, E., Nishino, T., Imagawa, K., Kumahara, Y., and Yanaihara, N. : Heterogeneity of plasma motilin in patients with chronic renal failure. Horm. Metab. Res. 12 : 328, 1980
46. Berson, S.A., Yalow, R.S., Bauman, A., Rothschild, M.A. and Newerly, K. : Insulin- ^{131}I metabolism in human subjects : Demonstration of insulin binding globulin in the circulation of insulin-treated subjects. J. Clin. Invest., 35 : 170, 1956
47. Berson, S.A., and Yalow, R.S. : Quantitative aspects of reaction between insulin and insulin-binding antibody. J. Clin. Invest., 38 : 1996, 1959
48. Yalow, R.S., and Berson, S.A. : Immunoassay of endogenous plasma insulin in man. J. Clin. Invest., 39 : 1157 1960
49. Yalow, R.S., and Berson, S.A. : Immunologic aspects of insulin. AM. J. Med., 31 : 882, 1961
50. Berson, S.A., and Yalow, R.S. : General radioimmunoassay. In : Methods in Investigative and diagnostic endocrinology, Part 1 - General Methodology, edited by S.A. Berson, and R.S. Yalow, p84-120. North-Holland Publishing Co., Amsterdam
51. Hunter, W.M., and Greenwood, F.C. : Preparation of Iodine - ^{131}I labelled human growth hormone of high specific activity. Nature (Lond.), 194 : 495, 1962
52. Thorell, J.I., and Johansson, B.G. : Enzymatic iodination of polypeptides with I^{125} to high specific activity. Biochem. Biophys. Acta, 251 : 363, 1971
53. Arimura, A., Sato, H., Coy, D.H., Worobec, R.B., Schally, A.V., Yanaihara, N., Hashimoto, T., Yanaihara, C. and Sukura, N. : The antigenic determinant of the LH-releasing hormone of three different antiserums. Acta Endocrinol. (Kbh), 78 : 222, 1975
54. Niswender, G.D., and Midgley, A.R. : Hapten-radioimmunoassay for steroid hormones. In : Immunological Method in Steroid Determination, edited by F.G. Peron and B.L. Caldwell P.149-166. Appleton-Century-Crofts, New York

55. Goodfriend, T.L., Levine, L. and Fasman, G.D. : Antibodies to bradykinin and angiotensin : a use of carbodiimides in immunology. *Science*, 144 : 1344, 1964
56. Hurn, B.A.L., and Landon, J. : Antisera for radioimmunoassay. pl21-142 in Kirkham, K.E. and Hunter, W.M. (eds) *Radioimmunoassay methods*. Churchill Livingstone, Edinburgh & London, 1971
57. Mitchison, N.A. : The carrier effect in the secondary response to hapten-protein conjugates. I. Measurement of the effect with transferred cells and objections to the local environment hypothesis. *Europ. J. Immunol.*, 1 : 10, 1971
58. Freund, J. : The effect of paraffin oil and mycobacteria on antibody formation and sensitization *AM J. Clin. Path.*, 21 : 645, 1951
59. Morgan, C.R., and Lazarow, A. : Immunoassay of insulin using a two antibody system. *Proc. Soc. Exp. Biol. Med.*, 110 : 29, 1962
60. Morgan, C.R. and Lazarow, A. : Immunoassay of insulin : Two antibody system. Plasma insulin levels of normal subdiabetic and diabetic rats. *Diabetes*, 12 : 115, 1963
61. Herbert, V., Lau, K.S., Gottlieb, C.W., and Bleicher, S.J. : Coated charcoal immunoassay of insulin. *J. Clin. Endocr. Metab.*, 25 : 1375, 1965
62. Buchanan, K.D. and McCarroll, A.M. : Comparison of methods of separation of free from bound hormone in the radioimmunoassay of insulin and glucagon. p266-272 in Kirkham, K.E. and Hunter, W.M. (eds) *Radioimmunoassay methods*. Churchill Livingstone, Edinburgh & London, 1971
63. Rehfeld, J.F., Stadil, F., and Rubin, B. : Production and evaluation of antibodies for the radioimmunoassay of gastrin. *Scand. J. Clin. Lab. Invest.*, 30 : 221, 1972
64. Rosengquist, G.L. and Holmquist, A.M. : The specificity of antibodies directed to porcine gastrin. *Immunochemistry*, 11 : 489, 1976
65. Chard, T. : The extraction and concentration of hormones from biological fluids. p491-502 in Kirkham, K.E. and Hunter, W.M. (eds) *Radioimmunoassay methods*. Churchill Livingstone, Edinburgh & London, 1971
66. Axen, R., Porath, J., and Ernback, S. : Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature*, 214 : 1302, 1967

67. Atassi, M.Z. : Chemical modification and cleavage of proteins and chemical strategy and immunochemical studies of proteins. In : *Immunochemistry of Proteins*, edited by M.Z. Atazzi, pl-161. Plenum Press, New York
68. Berson, S.A., and Yalow, R.S. : Immunoassay of protein hormones. In : *The Hormones*, Vol IV, p557. Pincus G. Thimann, K.V. and Astwood, E.B. (eds). New York Academic Press
69. Fahrenkrug, J., Schaffalitzky de Muckadell, O.B., and Rehfeld, J.F. : Production and evaluation of antibodies for radioimmunoassay of secretin. *Scand. J. Clin. Lab. Invest.*, 36 : 281, 1976
70. Scatchard, G. : The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, 51 : 660, 1949
71. Stadil, F. and Rehfeld, J.F. : Preparation of I-125 labelled synthetic human gastrin for radioimmunoanalysis. *Scand. J. Clin. Lab. Invest.*, 30 : 361, 1972
72. Laurent, T.C. and Killander, J. : A theory of gel filtration and its experimental verification. *J. Chromatogr.*, 14 : 317, 1964
73. Grossman, M.I. : Candidate hormones of the gut. *Gastroenterology*, 67 : 730, 1974
74. Lee, K.Y., Chey, N.Y., Tai, H.H., and Yajima, H. : Radioimmunoassay of motilin : Validation and studies on the relationship between plasma motilin and interdigestive myoelectric activity of the duodenum of dog AM. *J. Dig. Dis.*, 23 : 789, 1978
75. Dryburgh, J.R. and Brown, J.C. : Radioimmunoassay for motilin. *Gastroenterology* 68 : 1169-1176
76. Track, N.S., Watters, L.M. and Gauldie, J. : Motilin, human pancreatic polypeptide and gastrin plasma concentrations in fasting subjects. *Clin. Biochem.* 12 : 109, 1979
77. Yanaihara, C., Sato, H., Yanaihara, N., Naruse, S., Forsmann, W.G., Helmstaedter, V., Fujita, T., Yamaguchi, K. and Abe, K. : Motilin, substance P and somatostatin like immunoreactivities in extracts from dog, tupaia and monkey brain and GI tract. In : *Gastrointestinal Hormones and Pathology of the Digestive System*, edited by M. Grossman, V. Speranza, N. Basso, and D. Lezoche. Plenum Press, New York
78. Yanaihara, N., Yanaihara, C., Nagai, K., Sato, H., Shimizu, F., Yamaguchi, K. and Abe, K. : Motilin-like immunoreactivity in porcine, canine, human and rat tissues. *Biomed. Res.*, 1 : 76, 1980

79. Weir, G.C., Turner, R.C. and Martin, D.B. : Glucagon radioimmunoassay using antiserum 30K; interference by plasma. *Horm. metab. Res.* 5 : 241, 1973
80. Bryant, M.G. and Bloom, S.R. : Distribution of the gut hormones in the primate intestinal tract. *Gu.*, 20 : 653 1979
81. Chey, W.Y., Escoffery, R., Roth, F., Chang, T.M., You, C.H. and Yajima, H. : Motilin-like immunoreactivity in the gut and neurons of peripheral and central nervous system. *Reg. Pep. (Supplement 1)*, 1 : 19, 1980 (abstact)
82. Brown, J.C. and Dryburgh, J.R. : Isolation of motilin. In : *Gut Hormones*, edited by S.R. Bloom, Churchill Livingstone Press
83. Mitznegg, P., Domschke, W., Wunsch, E., Bloom, S.R., Domschke, S. and Demling, L. : Release of motilin after duodenal acidification. *Lancet*, 1 : 888, 1976
84. Collins, S.M., Lewis, T.P., Track, N., Fox, J. and Daniel, E.E. : Release of motilin. *Gastroenterology*, 74 : 1020, 1978 (Abstr)
85. Modlin, I.M., Mitznegg, P. and Bloom, S.R. : Motilin release in the pig. *Gut*, 19 : 399, 1978
86. Vantrappen, G., Janssens, J., Hellemans, J., and Ghoo, Y. : The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine. *J. Clin. Invest.*, 59 : 1158, 1977
87. Schoon, I.M., : The effects of antral distension on gastric acid secretion and on the release of gastrointestinal hormones in man. *Acta Physiol. Scand.*, Supplement 482 : 1, 1980
88. Mori, K., Seino, Y., Itoh, Z., Yanaihara, N., and Imura, H. : Motilin release by intravenous infusion of nutrients and somatostatin in conscious dogs. *Reg. Pep.*, 1 : 265, 1981
89. Track, N.S., Fox, J.E.T. and Daniel, E.E. : Motilin and motility in different species. *Reg. Pep.*, 1 : 116, 1980
90. Collins, S.M., Lewis, T.D., Fox, J.E.T., Track N.S. and Daniel, E.E. : Motilin release. Submitted for publication
91. Christofides, N.D. and Bloom, S.R. : Motilin. In : *Bloom, S.R., Polak, J.M. (eds). Gut Hormones*, 2nd edition. Churchill Livingstone, Edinburgh, 273, 1981

92. Polak, J.M. and Bloom, S.R. : The neuroendocrine design of the gut. In : Clinics in Endocrinology and metabolism, vol 8, No 2, edited by K. Buchanan., W.B. Saunders, London. P313, 1979
93. Polak, J.M., Ghatei, M.A., Wharton, J., Bishop A.E., Bloom, S.R., Solcia, E., Brown, M.R. and Pearse, A.G.E. : Bombesin-like immunoreactivity in the gastrointestinal tract, lung and central nervous system. Scand. J. Gastroent. (Suppl. 49), 13 : 148, 1978
94. Melchiorri, P.:- Bombesin-like peptides activity in the gastrointestinal tract of mammals and birds. In : Gastrointestinal Hormones, G.B.J. Glass (ed). Raven Press, New York. P717, 1980
95. Schusdziarra, V. : Somatostatin - A regulatory modulator connecting nutrient entry and metabolism. Horm. Metab. Res. 12 : 563, 1980
96. Adrian, T.E., Bloom, S.R., Besterman, H.S., Barnes, A.J., Cook, T.J.C., Russel, R.C.C., and Faber, R.G. : Mechanism of pancreatic polypeptide release in man. Lancet, : 1 : 161, 1977
97. Floyd, J.C., Jr., Fajans, S.S. Pek, S. and Chance, RE : A newly recognised pancreatic polypeptide : Plasma levels in health and disease. Recent Progr. Horm. Res., 23 : 519, 1977
98. Rehfeld, J.F. : Heterogeneity of gastrointestinal hormones. In : Gastrointestinal Hormones. G.B.J. Glass (ed). Raven. Press, New York. P433, 1980
99. Liljenquist, J.E., Chiasson, J.L., Jennings, A.S., Horwitz, D.L., and Rubenstein, A.H. : Insulin suppression of insulin secretion in man : demonstration by C-peptide assay. Diabetes (Suppl. 24), 2 : 403, 1975
100. Service, F.J., Nelson, R.L., Rubenstein, A.H. and Go, V.L.W. : Direct effect of insulin on secretion of insulin, glucagon, gastric inhibitory polypeptide and gastrin during maintenance of normoglycaemia. J. Clin. Endocrinol. Metab., 47 : 488, 1978
101. Moody, A.J. and Sundby, F. : Radioimmunoassay of gut glucagon-like immunoreactants. In : Gastrointestinal Hormones. G.B.J. Glass (ed). Raven Press, New York. P831, 1980
102. Hytten, F.E. and Leitch, I. (eds) : In : The Physiology of Human Pregnancy. Second edition. Blackwell Scientific Publications, 1971
103. Davison, J.S., Davison, M.G. and Hay, D.M. : Gastric emptying time in late pregnancy and labour. J. Obstet. Gynaec. Brit. Commonw., 77, 37, 1970

104. Castro, L.P. : Reflux oesophagitis as the cause of heartburn in pregnancy. Amer. J. Obstet. Gynec. 98 : 1, 1967
105. Gerdes, M.M., and Boyden, E.A. : The rate of emptying of the human gall-bladder in pregnancy. Surg. Gynec. Obstet., 66 : 145, 1938
106. Huggins, R.R., Harden, B. and Grier, G.W. : A study of the relationship of pregnancy to disease of the gall bladder. Surg. Gynec. Obstet., 61, 471, 1935
107. Christofides, N.D. : Importance of the jejunal hormone motilin. J. Clin. Path. (Suppl. 33), 8 : 51, 1980
108. Mitznegg, P., Domschke, W., Schubert, E., Domschke, W., Sprugel, W., Wunsch, E. and Demling, L. : Age dependence in fasting plasma motilin : elevation in infants. Gastroenterology, 72 : 1103
109. Carlson, A.J. and Ginsburg, H. : The tonus and hunger contractions of the stomach of the newborn. AM. J. Physiol. 38 : 29, 1915
110. Smith, C.A. : Physiology of the digestive tract. In : Smith, C.A. and Nelson, N.M. (eds). The physiology of the newborn infant. Springfield, Illinois : Thomas, P459, 1976
111. Cowley, D.J., Vernon P. and Jones, T. : Gastric emptying of solid meals after truncal vagotomy and pyloroplasty in human subjects. Gut, 13 : 176, 1972
112. Russell, R.C.G., Bloom, S.R., Fielding, R.P. and Bryant, M.G. : Current problems in the measurement of gastrin release. A reproducible measure of physiological gastrin release. Postgrad. Med J. 52 : 645, 1976
113. Adrian, T.E., Bloom, S.R., Bryant, M.G., Polak, J.M., Heitz, P.H., and Barnes, A.J. : Distribution and release of human pancreatic polypeptide. Gut, 17 : 940, 1976
114. Sarson, D.L., Bryant, M.G. and Bloom S.R. : A radioimmunoassay for gastric inhibitory polypeptide (GIP) in human plasma. J. Endocrinol., 85 : 487, 1980
115. Alford, F.P., Bloom, S.R. and Nabarro J.D.N. : Glucagon levels in normal and diabetic subjects : use of a specific immunoabsorbent for glucagon radioimmunoassay. Diabetologia, 13 : 1, 1977
116. Ghatei, M.A. and Bloom, S.R. : Enteroglucagon in man. In : Bloom, S.R., and Polak, J.M. (eds). Gut Hormones, 2nd Edn. Churchill Livingstone, Edinburgh, P 332, 1981

117. Mitchell, S.J. and Bloom, S.R. : Measurement of fasting and postprandial plasma VIP in man. *Gut*, 19 : 1043, 1978
118. Albano, J.D.M., Ekins, R.P., Maritz, G. and Turner, R.C. : A sensitive precise RIA of serum insulin relying on charcoal separation of bound and free hormone moieties. *Acta Endocrinol. (Kbh)*, 70 : 487, 1972
119. Hoffman, W.S. : Rapid photoelectric method for determination of glucose in blood and urine. *J. Biol. Chem.*, 120 : 51, 1937
120. Goldstein, A., Aronow, L. and Ralman, S.M. : Principles of drug action. Second edition. New York, John Wiley and Sons, P311, 1974
121. Saifer, A. and Gerstenfeld, S. : The photometric microdetermination of blood glucose with glucose oxidase. *J. Lab. Clin. Med.*, 51 : 448, 1958
122. Beaumont, W. : Experiments and Observations on the Gastric Juice and the Physiology of Digestion. Plattburgh : F.P. Allen, 1833
123. Hunt, J.N. and Knox, M.T. : A relation between the chain length of fatty acids and the slowing of gastric emptying. *J. Physiol. (Lond.)*, 194 : 327, 1968
124. Hunt, J.N. and Knox, M.T. : The effects of acids on gastric emptying. *J. Physiol (Lond)*, 181 : 63, 1965
125. Hunt, J.N. and Pathak, J.D. : The osmotic effects of some simple molecules and ions on gastric emptying. *J. Physiol. (Lond)*, 154 : 254, 1960
126. Hunt, J.N. and MacDonald, I. : The influence of volume on gastric emptying *J. Physiol (Lod)*, 126 : 459, 1954
127. Wolf, S., Wolff, H.G. : Human gastric function : an experimental study of a man and his stomach. New York, Oxford University Press, 1943
128. Chey, W.Y., Hitanant, S.M., Hendricks, J. and Lorber, S.H. : Effect of secretin and cholecystokinin on gastric emptying and gastric secretion in man. *Gastroenterology*, 58 : 820, 1970
129. Debas, H.T., Farooq, O. and Grossman, M.I. : Inhibition of gastric emptying is a physiological action of cholecystokinin. *Gastroenterology*, 68 : 1211, 1975

130. Hamilton, S.G., Sheiner, H.J. and Quinlan, M.F. : Continuous monitoring of the effect of pentagastrin on gastric emptying of solid food in man. *Gut*, 17 : 273, 1976
131. Hunt, J.N. and Ramsbottom, N. : Effect of gastrin II on gastric emptying and secretion during a test meal. *Br. Med. J.*, 4 : 386, 1967
132. Mitznegg, P., Bloom, S.R., Domschke, W., Domschke, S., Wunsch, E. and Demling, L. : Pharmacokinetics of motilin in man. *Gastroenterology*, 72 : 413, 1977
133. Szurszewski, J.H. : A migrating electric complex of the canine small intestine. *AM. J. Physiol.*, 217 : 1757, 1969
134. Chey, W.Y., Lee, K.Y. and Tai, H.H. : Endogenous plasma motilin concentration and interdigestive myoelectrical activity of the canine duodenum. In : Bloom, S.R. (ed). *Gut Hormones*. First edn. Churchill Livingstone, P355, 1979
135. Marik, F. and Code, C.F. : Control of the interdigestive myoelectric activity in dogs by the vagus nerves and pentagastrin. *Gastroenterology*, 69 : 387, 1975
136. Ormsbee, H.S., Telford, G. and Mason, R. : Required neural involvement in control of canine migrating motor complex. *AM. J. Physiol.*, 237 : E451, 1979
137. Carlson, G.M., Bedi, B.S. and Code, C.F. : Mechanism of propagation of intestinal interdigestive myoelectric complex. *AM. J. Physiol.*, 222 : 1027, 1971
138. Weisbrodt, N.W., Copeland E.M., Moore, E.P. Kearly, R.W. and Johnson, L.R. : Effect of vagotomy on electrical activity of the small intestine of the dog. *AM. J. Physiol.*, 228 : 650, 1975
139. Bueno, L. and Ruckebusch, Y. : Evidence for a role of endogenous insulin on intestinal motility. *Proceedings of the Fifth International Symposium on Gastrointestinal motility*, Vantrappen, G., (ed), Herentals, P64, 1976
140. Janssens, J., Vantrappen, G. and Hellemans, J. : The interdigestive myoelectric complex in man : Effect of food and high serum gastrin levels. *Gastroenterology*, 72 : 1161, 1977
141. Hellemans, J., Vantrappen, G., Janssens, J., and Peeters, T. : Effects of feeding and of gastrin on the interdigestive myoelectric complex in man. *Proceedings of the VIth International Symposium on Gastrointestinal Motility*, Duthie, H., (ed). Edinburgh, MTP Press, P 29, 1978

142. Borody, T., Byrnes, D., Slowiaczek, J. and Titchen, D.: Effect of motilin antiserum infusion on porcine IDMCS. Reg. Pep. (Suppl. 1), 1 : 13, 1981
143. Lux, G., Lederer, P., Femppel, J., Rosch, W. and Domschke, W. : Somatostatin induces interdigestive intestinal motor and secretory complex-like activity in man. Reg. Pep. (Suppl. 1), 1 : 71, 1981
144. Poitras, P., Steinbach, J.H., VanDeventer, G., Code, C.F. and Walsh, J.H. : Motilin-independent ectopic fronts of the interdigestive myoelectric complex in dogs. AM. J. Physiol., 2 : G215, 1980
145. Krejs, G.J. and Fordtran, J.S. : Diarrhoea. In : Sleisenger, M.H. and Fordtran, J.S. (eds). Gastrointestinal Disease, Second edn. W.B. Saunders company, P313, 1978
146. Waller, S.L. and Misiewicz, J.J. : Colonic motility in constipation or diarrhoea. Scand. J. Gastroent. 7, 93-96
147. Rennie, J.A., Christofides, N.D., Mitchener, P., Johnson, A.G. and Bloom, S.R. : Motilin and human colonic activity. Reg. Pep., (Suppl 1), 1 : 92, 1980
148. Christofides, N.D. and Bloom, S.R. : Plasma motilin levels in duodenal ulcer and effect of truncal vagotomy and hypoglycaemia. Experientia (Basel), 34 : 809, 1978
149. Besterman, H.S., Sarson, D.L., Hsu, G., Crisp, A.H. and Bloom, S.R. : Gut hormones in thyrotoxicosis and anorexia nervosa. J. Endocr., 81 : 143, 1979
150. Long, R.G., Albuquerque, R.H., Prata, A., Barnes, A.J., Adrian, T.E., Christofides, N.D. and Bloom, S.R. : Response of plasma pancreatic and gastrointestinal hormones and growth hormone to oral and intravenous glucose and insulin hypoglycaemia in Chagas's disease. Gut, 21 : 772, 1980
151. Blackburn, A.M., Christofides, N.D., Ghatei, M.A., Sarson, D.L., Ebeid, F.H., Ralphs, D.N.L. and Bloom, S.R. : Elevation of plasma neurotensin in the dumping syndrome. Clin. Sci., 59 : 853, 1980
152. Bloom, S.R., Besterman, H.S., Adrian, T.E., Christofides, N.D., Sarson, D.L., Mallinson, C.N., Pera, A., South, M., Modigliani, R. and Guerin, S. : Gut hormone profile in pancreatic disease. Gastroenterology, 74 : 1012 1978

153. Mallinson, C.N. : Effect of pancreatic insufficiency and intestinal lactase deficiency on the gastric emptying of starch and lactose. Gut, 9 : 737, 1968

154. Rennie, J.A., Christofides, N.D., Mitchenere, P., Fletcher, D., Leathard, H., Bloom, S.R., Johnson, A.G. and Harding-Rains, A.J. : Neural and humoral factors in post-operative ileus. Br. J. Surg., 67 : 694, 1980