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Gastric intrinsic factor (IF) secreted by the stomach of mammals is a glycoprotein which binds to vitamin B_{12}, and facilitates its absorption from the intestine. The cells responsible for the secretion of IF vary according to the animal studied. In the ruminant little is known about the site of production and the secretion of IF. In the present experiments tissues of the sheep stomach and intestine were examined by fluorescent protein tracing procedures to determine the location and identification of the cells responsible for the secretion of IF and the secretion of IF in the gastric juice of sheep was compared with that of humans.

Identification of the cells secreting IF by immunofluorescence was based on an indirect sandwich technique (Coons *et al.*, 1955). Tissues were studied utilizing specific autoantibodies directed to cell mitochondria, to a parietal cell canalicular antigen and to IF. The antimitochondrial antibody was used as a reactive control and was obtained from human patients with primary biliary cirrhosis. The antiparietal cell canalicular antibody was obtained from human patients with chronic atrophic gastritis without pernicious anaemia. The IF antibody was obtained from a human patient with juvenile pernicious anaemia. The specificity of the antibodies was tested by prior absorption procedures utilizing porcine fundic and ovine kidney homogenates and purified IF (Radiochemical Centre, Amersham, England). Fluorescence labelling was provided by goat antihuman immunoglobulins conjugated to fluorescein isothiocyanate (Behringiverke Laboratories, Marburg, Germany). Tissues of the reticulum, rumen, omasum antral and body regions of the abomasum and duodenum were obtained within 2 h of slaughter, cut into 5-6 mm cubes and snap frozen in liquid nitrogen. Sections 6μ thick were cut on a cryostat and mounted on a glass slide, dried and the appropriate antibody containing serum applied. After 30 min the preparation was washed with phosphate buffered saline, dried and conjugated fluorescent label added. Thirty minutes later the preparation was washed, dried, mounted in glycerol and examined with a Reichert Immunopan microcope with a dark field condenser and using a quartz iodine light source.

Gastric secretion from separated fundic pouches of the body of the abomasum of three sheep fed *ad libitum* was collected at 15 minute intervals for 4 h and at 24 h intervals (McLeay and Titchen, 1974). Gastric secretion of three humans was collected by stomach aspiration at 15 minute intervals for 90 minutes following stimulation with 6μg. kg $^{-1}$ pentagastrin (Peptavlov, ICI, England). The secretions were brought to pH7-8 with 10M NaOH and the IF concentration estimated by radioimmu-

noassay (Ardeman and Chanarin, 1963) using ^{57}Co-Vitamin B_{12} (Radiochemical Centre, Amersham, England).

All tissues showed strong fluorescence to the antimitochondrial antibody. Parietal cells in the fundic region of the abomasum were the only cells to react positively with the antiparietal cell canalicular antibody. The anti IF antibody revealed positive fluorescence in the fundic region of the abomasum only and the cells reacting corresponded to parietal cells as revealed by the previous antimitochondrial and antiparietal cell antibody experiments. Fluorescence was not obtained from any tissue when absorbed, neutral human AB serum replaced specific antibody containing serum.

Fundic pouch secretion of sheep possessed IF in relatively constant concentrations. Secretion collected at 15 minute intervals had an IF concentration of 3.87 $\pm$ 0.13 I.U. ml $^{-1}$ (mean $\pm$ sem, n = 8) and in 24 h collections the concentration was 3.62 $\pm$ 0.12 I.U. ml^{-1} (n = 9). In contrast IF in gastric juice of 3 humans before stimulation by pentagastrin was 3.2 -8.9 I.U. ml^{-1} and reached maximal concentrations of 11.5-38.7 I.U.ml^{-1} within 15 minutes of pentagastrin administration. In two sheep the abomasal pouches were 15 and 25 % of the surface area of the body of the abomasum and it was estimated that the total abomasal secretion of IF was 10,400 - 23,500 I.U. 24 h^{-1}.

It is concluded that the fluorescence studies show that the abomasal parietal cell is the site of IF secretion in the sheep and this is supported by the presence of IF in pure fundic pouch secretion. The uniform and relatively low concentrations of IF found in fundic secretion of sheep is consistent with the continuous nature of abomasal secretion and presumably parietal cell stimulation.

References

ARDEMAN S., CHANARIN I., 1963. A method for the assay of human gastric intrinsic factor and for the detection and titration of antibodies against intrinsic factor. *Lancet 2,* 1350-1354.

COONS A.H., LEDUC E.H., CONNOLY J.M., 1955. Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J. Exp. Med.,* **102**, 49-60.

McLEAY L.M., TITCHEN D.A., 1974. Effects of the amount and type of food eaten on secretion from fundic abomsal pouches of sheep. *Br. J. Nutr.,* **32**, 375-387.