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Demonstration of human apolipoprotein A in isolated mucosal cells from small intestine and isolated hepatocytes

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SUMMARY Isolated mucosal cells from the human jejunum and stomach, cryostat sections from the jejunum, isolated parenchymal liver cells and lymphocytes were investigated for the presence of apolipoprotein A (apoA). Antisera against purified human apoA-I and apoA-II were raised in rabbits and conjugated with fluorescein-isothiocyanate (FITC). Mucosal cells from jejunum and stomach were isolated with pronase from tissue obtained from operated patients. ApoA-I and apoA-II could be demonstrated in isolated mucosal cells as well as in cryostat sections from the jejunum. The fluorescence pattern in isolated jejunal cells was coarse granular. In the radial gel diffusion test the homogenate from mucosal cells of jejunum showed a single precipitation line with anti-apoA-I and with anti-apoA-II, respectively. The reaction was more intensive with anti-apoA-I than with anti-apoA-II. Isolated gastric cells were negative for apoA. Hepatocytes incubated with FITC anti-apoA-I showed a fine granular fluorescence pattern in the cytoplasm. Anti-apoA-II did not react with hepatocytes. There was no evidence for an *in vitro* fixation of serum-apoA at the surface of isolated mucosal cells from jejunum or isolated hepatocytes. The results support the hypotheses that in man apoA is synthesised in the epithelial cells of the small intestine and in parenchymal liver cells.

Apoprotein A-I (apoA-I) and apoprotein A-II (apoA-II) are the predominant components of the human high density lipoproteins (HDL). The HDL have an essential importance in the transport and metabolism of serum cholesterol. Biochemical, clinical, and epidemiological data have been accumulated to support the specific role of HDL in atherogenesis (Fredrickson and Levy, 1972; Karlin *et al.*, 1976; Gordon *et al.*, 1977; Cheung and Albers, 1977). However, tissue sites of synthesis and degradation of HDL and factors controlling their plasma concentrations are largely unknown. Studies in animals have suggested that HDL are secreted in the mucosa of the small intestine and the liver (Roheim *et al.*, 1966; Hamilton, 1972; Windmueller and Spaeth, 1972; Glickman and Green, 1977; Schonfeld *et al.*, 1977; Green *et al.*, 1978).

The present investigations are concerned with the detection of apoA-I and apoA-II in isolated mucosal cells from the small intestine as well as in isolated hepatocytes from man. Moreover, the cells were incubated in human serum for binding studies of apoA-I and apoA-II on the plasma membrane.

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Methods

PREPARATION OF APOA-I AND APOA-II

The human apoA was prepared from plasma HDL. The procedure is described in detail elsewhere (Assmann *et al.*, 1977).

PREPARATION OF ANTISERA

One to two milligram amounts of apoA-I or apoA-II in 1 ml H_2O were emulsified in an equal amount of complete Freund's adjuvant (Difco Lab., Detroit, Mich.). Rabbits were injected subcutaneously on three occasions 14 days apart and bled seven days after the last injection. The antisera were tested by double immunodiffusion and immunoelectrophoresis against different antigens and appeared to be monospecific. The antisera were conjugated with FITC according to the method described elsewhere (Arnold and Mayersbach, 1972).

TISSUE MATERIAL

Tissue from small intestine and stomach was obtained from five patients operated on for an ulcer. The tissue was harvested in Eagle's medium. The preparation of cells was performed at the same day. Liver biopsies were performed in three patients with histologically normal livers, who participated in a controlled study and had recovered from an acute virus hepatitis one year ago, as well as in eight patients with fatty liver.

ISOLATION OF MUCOSAL CELLS

A modification of the method described by Blum *et al.* (1971) was used. For each preparation 20-50 cm^2 mucosa were available. The superficial layer of the mucosa was scraped off by a scalpel. The material so obtained was incubated in 50 ml 0·175% pronase (Merck Lab., Darmstadt, Germany, 70·000 protease units per mg) in Eagle's medium for two hours at 30°C in a shaking water bath (100 cycles per minute). The cell suspension was washed three times in Eagle's medium (centrifugation 60 *g*, five minutes). The isolated surface cells were morphologically intact and retained morphological characteristics of the plasma membrane. About 95% of the isolated cells excluded trypan blue. Moreover, suspensions with isolated jejunal mucosal cells were dropped on a slide, air-dried, and stained according to Pappenheim, with Sudan black (Schaefer and Fischer, 1972) and Astra blue. Alkaline phosphatase was also demonstrated cytochemically (Kaplow, 1955).

CRYOSTAT SECTIONS

Jejunal tissue was stored immediately in liquid N_2 until the moment of use. Slices of 4 and 6 μ thickness were obtained from a cryostat and placed on a microscope slide.

ISOLATION OF HEPATOCYTES

Human hepatocytes were isolated without enzymes from biopsy material as described elsewhere (Hopf *et al.*, 1976).

ISOLATION OF LYMPHOCYTES

Human lymphocytes were isolated from heparinised peripheral blood by the method of Kissmeyer-Nielsen and Kjerbye (1967).

IMMUNOFLUORESCENCE TECHNIQUE

In a typical experiment 50 μl of isolated cells (ca. 2×10^5 cells in Eagle's medium) were incubated in 100 μl FITC-anti-apoA-I or FITC-anti-apoA-II (F/P ratio 3·6 and 3·1 respectively; protein concentration: 2 mg per ml; incubation time 30 minutes at 37°C). The cells were washed three times in Eagle's medium and resuspended in fresh medium. Additional unfixed cell suspension air-dried on a slide at room temperature was incubated with 100 μl FITC-anti-apoA-I or FITC-anti-apoA-II for 30 minutes at 37°C. After rinsing with PBS (pH 7, 5) for 15 minutes using three different cuvettes the preparation was covered with glycerol. The tissue slices were layered with FITC-labelled apoA-I or apoA-II antibodies, allowed to stand one-half hour in a humidity chamber at room temperature, then washed three times (five minutes each) in phosphate-buffered saline (0·15 M, pH 7, 5) and dried under a cold air stream. FITC-conjugated non-immune sera and antisera that had been absorbed with pure A apoproteins served as controls. Blocking experiments with unlabelled antisera were also performed. The immunofluorescence studies were also carried out with tissue slices that had been pretreated for four minutes with cold chloroform or cold chloroform/acetone (2:1, v/v). The preparations were viewed under a Leitz fluorescent microscope Orthoplan II adjusted to selective conditions for green fluorescence.

RADIAL GEL DIFFUSION

Pellets of isolated mucosal cells were homogenised by an ultrasonic homogeniser (10 seconds). 10 μl of the homogenate (ca. 5 mg protein per ml) were tested in the radial gel diffusion (Ouchterlony, 1958) against anti-apoA-I, anti-apoA-II, antihuman IgG, and antihuman IgM from rabbits. Antisera against human IgG and IgM were purchased from Behring-Werke, Marburg, Germany.

Results

LOCALISATION OF ALKALINE PHOSPHATASE

The air-dried preparations of isolated mucosal cells from the jejunum contained a few goblet cells demonstrable by the staining with Astra blue. However, the predominant cell type was positive for alkaline phosphatase on the plasma membrane and could be identified as resorptive epithelial cells (Fig. 1). The cells formed partially into balls and showed a mulberry-like surface structure. As a consequence of the treatment with pronase, the alkaline phosphatase, *in situ* restricted to the brush border (Fig. 2), showed a spreading over the whole rounded surface of the epithelial cell.

LOCALISATION OF APOA-I AND APOA-II

Intracellular apoA-I and apoA-II could be detected in isolated mucosal cells from the human small intestine but not in mucosal cells from the stomach (Table). The fluorescence pattern was coarse granular for both A apoproteins (Fig. 3). The intensity of

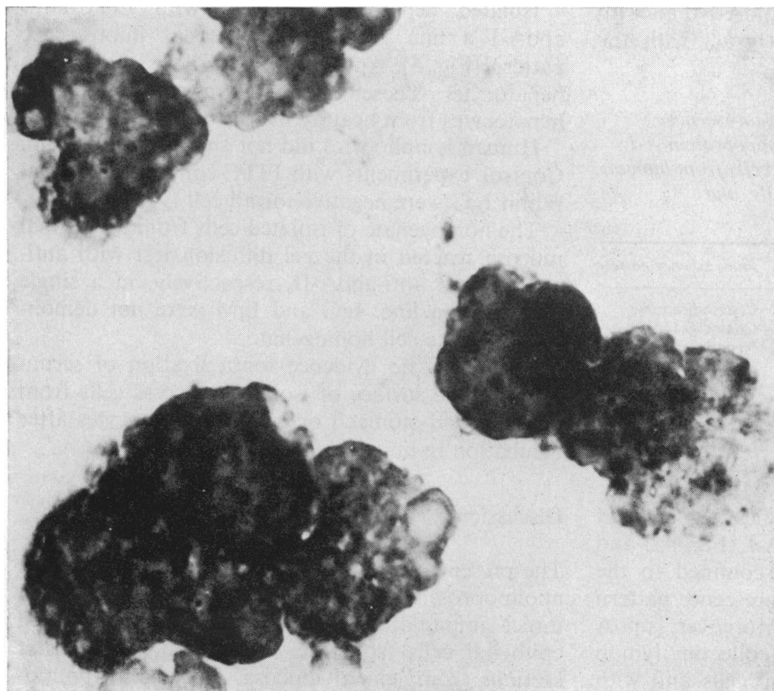

Fig. 1 *Demonstration of alkaline phosphatase (darker areas) on isolated mucosal cells from human jejunum by cytochemical reaction. The alkaline phosphatase is spread over the whole surface of the cells which show a mulberry-like picture after the treatment with pronase × 500.*

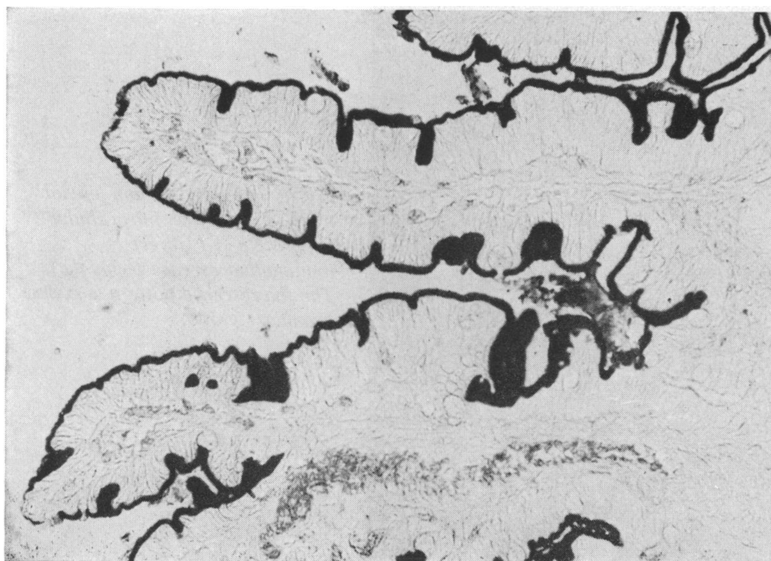

Fig. 2 *Histochemical staining of alkaline phosphatase on normal mucosa from human jejunum (after fixation with formalin and imbedded in paraffin). The alkaline phosphatase is restricted to the brush border. Original magnification × 240.*

fluorescence was higher with anti-apoA-I than with anti-apoA-II. Viable cells did not react with the antisera.

Table *Summarised results of immunofluorescence studies for detection of intracellular apolipoprotein A-I and A-II in isolated resorptive mucosal cells from human jejunum, stomach, parenchymal liver cells, and lymphocytes*

Cell type	*Incubated with*	*Fluorescence pattern*
Jejunal mucosa cells	FITC anti-apoA-I	Coarse granular
	FITC anti-apoA-II	Coarse granular
Stomach mucosa cells	FITC anti-apoA-I	Negative
	FITC anti-apoA-II	Negative
Hepatocytes	FITC anti-apoA-I	Fine granular
	FITC anti-apoA-II	Negative
Lymphocytes	FITC anti-apoA-I	Negative
	FITC anti-apoA-II	Negative

In the immunofluorescence studies on cryostat sections from jejunal mucosa apoA-I (Fig. 4a) and apoA-II (Fig. 4b) were primarily confined to the apex of the epithelial cells. The fluorescence pattern was impressed homogeneously, Moreover, apoA could be detected within the small collecting lymph vessels at the base of the epithelial cells and with larger lymph vessels in the jejunal stroma. All fluorescence was specifically blocked by prior absorption of the antisera with purified A apoproteins. Immunofluorescence was also completely eliminated by prior addition of unlabelled antibodies to the tissue slices.

Isolated hepatocytes showed with FITC-anti-apoA-I a fine granular intracellular fluorescence pattern (Fig. 5). ApoA-II could not be observed in hepatocytes. These findings were not different in hepatocytes from healthy liver or fatty liver.

Human lymphocytes did not show A apoprotein. Control experiments with FITC-conjugated normal rabbit IgG were negative for all cell types tested.

The homogenate of isolated cells from the jejunal mucosa reacted in the gel diffusion test with anti-apoA-I and anti-apoA-II, respectively, in a single precipitation line. IgG and IgM were not demonstrable in the cell homogenate.

There was no evidence for a fixation of serum apoA at the surface of isolated mucosal cells from jejunum and stomach or isolated hepatocytes after incubation in human serum.

Discussion

The present investigations demonstrate that human apolipoproteins A-I and A-II can be detected by the direct immunofluorescence technique in isolated epithelial cells from the jejunum and in cryostat sections from jejunal mucosa. The main apolipoprotein detectable in the epithelial cells of the jejunum seems to be apoA-I. The different patterns of A apolipoproteins in isolated cells (drop-shaped) and cryostat sections (confined to the apex of the cells) may be a consequence of the preparation because isolated mucosal cells alter their structure

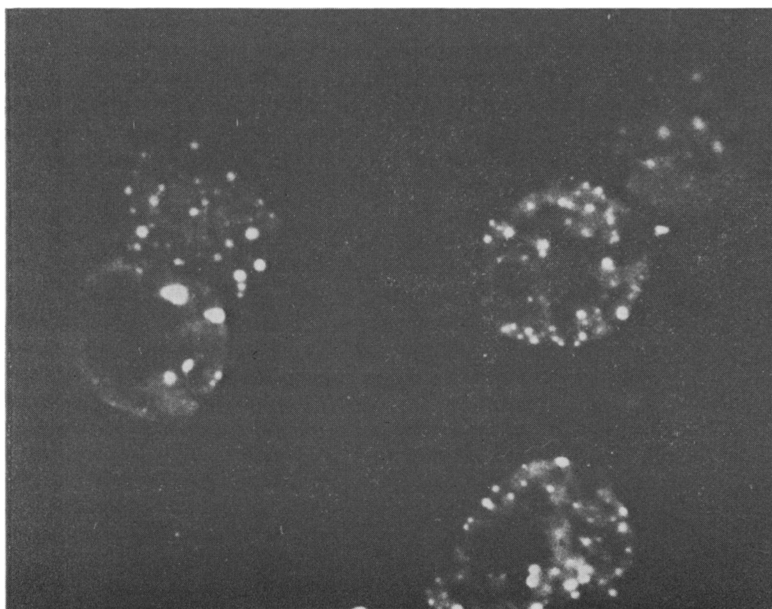

Fig. 3 *Isolated human jejunal mucosal cells with intracellular apoprotein A-I (direct immunofluorescence technique). The fluorescence pattern is coarse granular* × *500.*

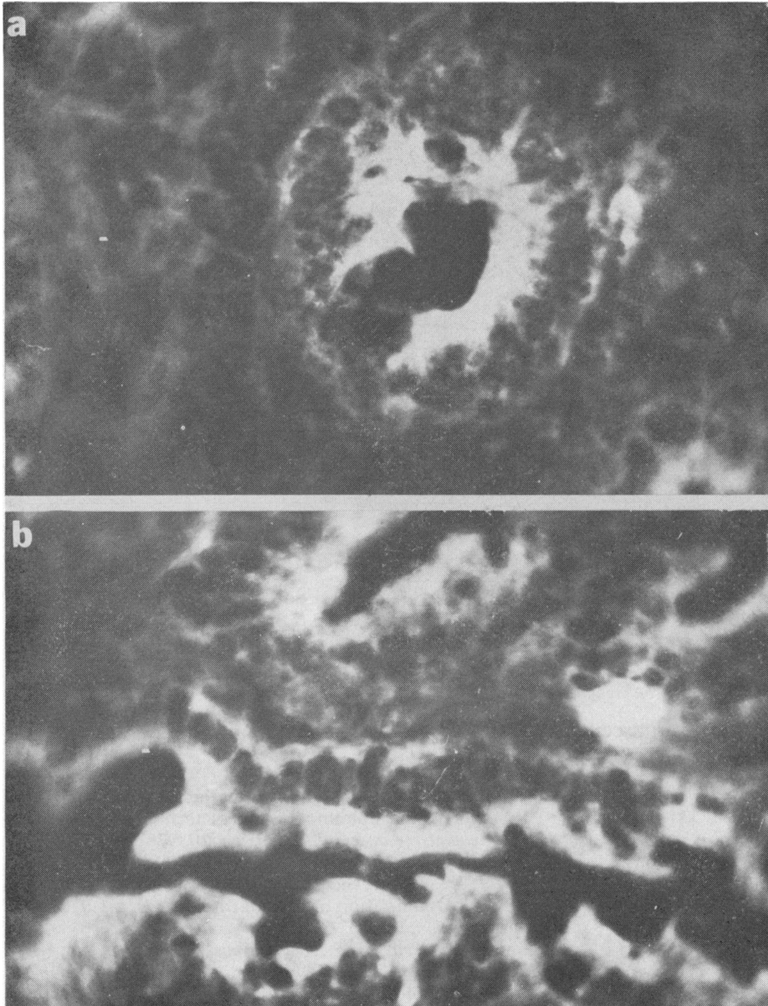

Fig. 4 *Direct apoA-immunofluorescence of human jejunal mucosa; cryostat section treated with FITC-conjugated antiserum against (a) apoA-I, and (b) apoA-II. Fluorescence mainly confined to the apex of epithelial cells × 350.*

in vitro. This could be demonstrated by the staining for alkaline phosphatase.

Isolated human hepatocytes show intracytoplasmatic apoA-I in a fine granular fluorescence pattern. ApoA-II was not demonstrable in parenchymal liver cells. This observation supports the hypothesis that hepatocytes contain and possibly synthethise less A apoproteins than mucosal cells from the small intestine. It can not be excluded that the intrahepatic apolipoprotein A is partially catabolised by parenchymal liver cells. However, a recent report has shown extrahepatic catabolism of apoA in rats (van Tol *et al.*, 1978). Gastric mucosa cells and lymphocytes did not contain immunochemically detectable amounts of A apoproteins, which is indirect evidence for the specificity of the demonstration of apoA in intestinal mucosa cells and hepatocytes. There was no evidence for an *in vitro* binding of serum apolipoproteins A-I and A-II on the plasma membrane of isolated mucosal cells from human jejunum or isolated hepatocytes after incubation in human serum. Therefore it is suggested that these cells do not possess membrane receptors for A apoproteins. However, the possible effect of pronase to the mucosal cells has to be considered.

Experiments in rats have provided evidence that the intestine and the liver actively synthesise nascent HDL which are secreted as disc-shaped particles (Hamilton *et al.*, 1976; Felker *et al.*, 1976; Schönfeld *et al.*, 1977; Green *et al.*, 1978). The intestinal formation of HDL was also demonstrated by the uptake of labelled amino acids in plasma HDL of hepatectomised rats (Roheim *et al.*, 1966) as well as in the model of isolated perfused rat intestine

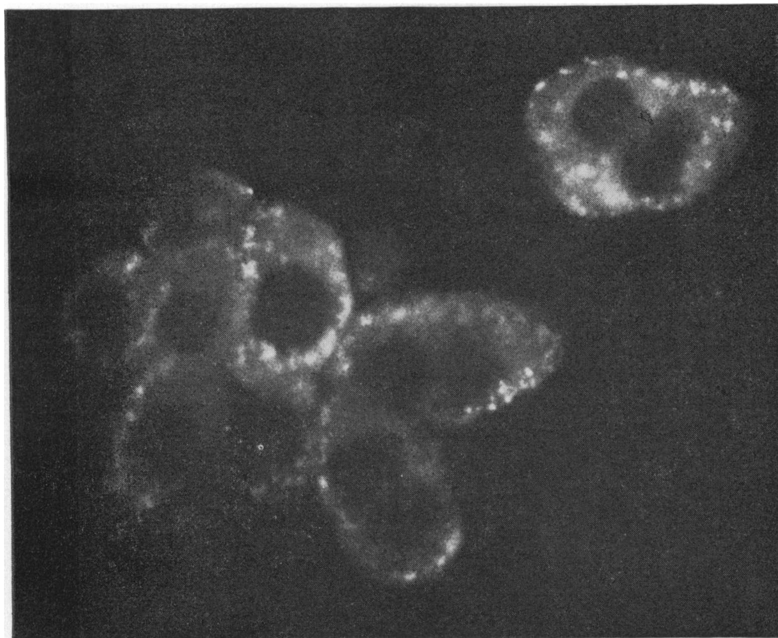

Fig. 5 *Isolated human hepatocytes with a fine granular fluorescence pattern of apoproteinA-I in the cytoplasm. Direct immunofluorescence technique × 500.*

(Windmueller and Spaeth, 1972). In the rat, nascent HDL is deficient in apoA-I (Hamilton *et al.*, 1976), suggesting that intestinal apoA-I is a significant source of this apoprotein for circulating HDL.

The origin of the A apoproteins in man is less well understood. It has been demonstrated that human chylomicrons from the thoracic duct lymph contain apoA-I (Kostner and Holasek, 1972) that possibly serve as precursor for plasma HDL (Schaefer *et al.*, 1978). Similarly, Rachmilewitz *et al.* (1977) report in abstract form on the synthesis of apoA-I in the human small intestine. On the basis of our experiments and data in the literature it can be assumed that a significant proportion of the human A apolipoproteins is synthesised in the gut and enters the plasma compartment *via* chylomicrons. Other studies in our laboratory are directed to correlate the intracellular presence of A apolipoproteins to subcellular structures.

Isolated mucosal cells from the human small intestine seem to be suitable for *in vitro* studies of the biosynthesis and regulatory mechanisms of human apolipoproteins.

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