uptake by mucosal biopsies of human duodenum (Cox & Peters, 1977, Gut, in press) the rate of radioiron 
$^{55}$Fe$^{3+}$ uptake at concentrations of 180 and 450 μmol/l into biopsies from patients with iron-deficiency anaemia was greatly increased when compared with controls (Table 1).

| Table 1 |
|-----------------|-----------------|-----------------|
| Uptake at 180 μmol/l | Uptake at 450 μmol/l |
| (pmol min$^{-1}$ mg$^{-1}$) | (pmol min$^{-1}$ mg$^{-1}$) |
| Controls | Fe deficient |
| 6.2 ± 1.5 SEM (Hb 13.9 ± 0.2 g/dl) | 11.9 ± 3.6 (Hb 8.8 ± 1.3 g/dl) |
| n = 6 | n = 3 |
| 9.2 ± 1.6 (Hb 13.8 ± 0.4 g/dl) | 24 ± 2.5* (Hb 8.8 ± 0.4 g/dl) |
| n = 8 | n = 4 |

*P < 0.01

In addition, we have shown that unidirectional influx of $^{55}$Fe$^{3+}$ radio-labelled iron into human duodenal mucosa is an energy

dependent, saturable process significantly reduced by metabolic inhibitors and having a $Q_{10}$ of approximately 3. These results are consistent with active transport of iron by the enterocyte.

Assay of total iron by atomic absorption spectrophotometry in the biopsies from patients with iron-deficiency anaemia showed significantly lower concentrations when compared with controls, 74 ± 16 SEM nmol of Fe/mg of protein and 150 ± 28 nmol of Fe/mg of protein respectively ($P < 0.05$).

Studies in iron deficiency, before and after 6 weeks treatment with oral iron (3.2 mmol/day) show suppression of the iron uptake, although the total iron content of the duodenal mucosa remained low. These data indicate that there is important control of iron absorption in man by regulation at the entry step into the enterocyte.

This work is supported by the Grocers Trust and the Medical Research Council.

24. SEPARATION AND QUANTIFICATION OF TRANSFERFERRIN, FERRITIN, HAEMPROTEINS AND HAEMOSIDERIN IN HEPATIC NEEDLE BIOPSY SPECIMENS FROM CONTROL SUBJECTS AND PATIENTS WITH IRON OVERLOAD

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Measurement of hepatic storage iron is a useful method to follow treatment of both primary and secondary iron overload. However, the nature of the iron compounds and the mechanisms of liver damage due to iron overload are ill understood.

Combining ion-exchange chromatography to separate the iron proteins (Drysdale & Ramsay, 1965, Biochemical Journal, 95, 282) with atomic absorption spectrophotometry, a method of quantifying the transferrin fraction, ferritin, haemproteins and haemosiderin has been established.

In nine specimens of control liver the levels of the iron compounds were (mean ± SE, μg of Fe/mg of tissue protein; percent iron distribution shown in parentheses): transferrin 0.490 ± 0.250 (27%); ferritin 0.659 ± 0.350 (47%); haemprotein 0.336 ± 0.120 (14%); haemosiderin 0.284 ± 0.110 (12%).

In a total of seven patients with either primary haemochromatosis (untreated) or transfusion-dependent thalassaemia major the levels of the iron compounds were: transferrin 0.204 ± 0.070 (0.5%); ferritin 2.93 ± 0.60 (12%); haemprotein 3.07 ± 0.94 (10.5%); haemosiderin 25.8 ± 6.0 (77%).

These studies indicate that the transferrin fraction is unaltered in iron overload: ferritin, haemprotein and haemosiderin are increased approximately 5-, 10- and 100-fold respectively. There were no qualitative or quantitative differences in the hepatic levels of the iron protein compounds in patients with either untreated primary haemochromatosis or transfusional haemosiderosis.

This work was supported by The Wellcome Trust and The Grocers Trust.

25. ORGANELLE PATHOLOGY AND ENZYME ACTIVITIES, WITH PARTICULAR REFERENCE TO ALKALINE PHOSPHATASE, IN NEUTROPHILS FROM PATIENTS WITH CHRONIC GRANULOCYTIC LEUKAEMIA

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Neutrophils from patients with chronic granulocytic leukaemia (CGL) characteristically have a low histochemical alkaline phosphatase score and show impaired microbial activities. In contrast, neutrophil alkaline phosphatase is elevated in the third trimester of pregnancy when microbial activity is normal.

The specific activities (milliunits/mg of protein) and subcellular distribution of alkaline phosphatase, and organelle marker enzymes, were determined in neutrophils from control subjects, pregnant women and patients with CGL (Segal & Peters, 1976, Clinical Science and Molecular Medicine, 50, 6). 5'Nucleotidase, a plasma membrane enzyme, was reduced eightfold in the CGL patients compared with the other two groups but had identical distributions in the three groups. The following enzymes had similar activities and distribution in the three groups: lactate dehydrogenase (cytosol); malate dehydrogenase (mitochondria); neutral α-glucosidase and γ-glutamyl transpeptidase (endoplasmic reticulum); vitamin B$_{12}$-binding protein (specific granules); myeloperoxidase (azurophil granules); lysozyme (specific and azurophil granules).

The levels of neutrophil alkaline phosphatase in the control, pregnant and CGL groups were 3.31 ± 0.57, 23.9 ± 4.98 and 0.43 ± 0.11 respectively, and the levels correlated ($r = 0.851$, $P < 0.01$) with the neutrophil alkaline phosphatase scores.

The apparent $K_{m}$ (mean 0.043, range 0.037–0.052 mmol/l), latent activity and subcellular distribution of alkaline phosphatase were identical in the three groups. Electron-microscopic histochemical studies confirmed that most of this enzyme was localized to the endoplasmic reticulum.

These studies have clearly shown an abnormality of the plasma membrane enzyme 5'-nucleotidase in CGL neutrophils which could be related to their impaired microbial activity.

We have confirmed the endoplasmic reticulum localization of alkaline phosphatase. It has been said that the low alkaline phosphatase in CGL is due to cell ageing but the levels of other enzymes that we have measured do not support this.

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26. THE DISTRIBUTION OF 5'-NUCLEOTIDASE ON HUMAN LYMPHOCYTES

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The plasma membrane ectoenzyme 5'-nucleotidase primarily breaks down adenosine monophosphate to adenosine. Its activity is low in most patients with 'common variable' hypogammaglobulinaemia and usually exceedingly low in patients with chronic lymphocytic leukaemia.