Protein loss and cell loss from the small-intestinal mucosa[1]

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SUMMARY   Total protein and deoxyribonucleic acid (DNA) were measured in small-intestinal washings from rats with normal and high rates of DNA (or cell) loss. There was a significant relationship between the loss of these two substances. Preparations of isolated viable epithelial cells contained much less protein in relation to DNA than did the intestinal washings. It was calculated that only 8 to 15% of the protein lost by washing the rat small intestine arose from the intracellular protein of exfoliated epithelial cells. The rest was derived from extracellular sources.

Protein and DNA loss from small-bowel mucosa was measured in six patients. The ratio of protein to DNA was similar to that found in the rats. The mean protein loss from 5 cm of human upper small intestine was 956 μg per min or 1·4 g per 24 hours. By calculation, total protein loss from the whole small intestine of man was about 84 g per day, about 10 g coming from within exfoliated cells and the rest arising from extracellular sources. The normal intestine must reabsorb most of this material.

The small bowel handles much more protein than the quantity taken by mouth. For instance, in the dog and rat, Nasset and Ju (1961) found that ingested protein was swamped four- or fivefold by endogenous protein. In man it has been estimated that some 45-140 grams of protein are lost into the gut per 24 hours (Spencer, 1960; Wilson, 1962). This large quantity of protein is derived in part from gastrointestinal secretion from saliva, stomach, pancreas, and bile (Spencer, 1960; Nasset, 1964). It has been suggested that exfoliated epithelial cells may be another significant source of endogenous protein (Nasset, 1964; Jefferies, Holman, and Sleisenger, 1962). Wilson (1962) claimed that small intestinal cells in man may account for 25 g of protein per 24 hours and Nasset (1965) calculated that the amount could be as much as 90 g.

The small-intestinal mucosa is dynamically the most active epithelial surface in the body, in man losing some 20-50 million cells per minute (Croft, Loehry, Taylor, and Cole, 1968b). If significant amounts of protein are lost in exfoliated cells the small bowel is an important organ to study this phenomenon. We have done so by measuring, in intestinal washings from rat and man, deoxyribo-

nucleic acid (DNA) loss (as an estimate of cell loss), and protein loss. To assess the contribution of intracellular protein intestinal epithelial cells were isolated and their DNA and protein content was measured.

Materials and Methods

BIOCHEMICAL METHODS
Deoxyribonucleic acid was measured by a modified diphenylamine reaction and the values were expressed in ng atoms DNA-P (Croft and Lubran, 1965).

Protein was measured by the Lowry method (Papadopoulos, Hess, O'Doherty, and McLane, 1959), the standard curve being made with human serum protein. Aliquots of the specimens were concentrated by dialysis. The final technique was highly sensitive and reproducible (da Costa, 1970).

WASHINGS OF RAT SMALL INTESTINE
The details of this technique have already been described (Loehry, Croft, Singh, and Creamer, 1969). Male albino rats weighing 350-450 g were starved for 24 hours and then anaesthetized. Two pieces of polyvinylchloride tubing were tied respectively into the jejunum at the duodenojejunal flexure and at the terminal ileum. Normal saline was infused via the jejunal tube at measured rates for two to four hours. Fluid was collected from the ileal tube. The rats were killed at the end of the experi-

ments. In 11 normal rats the small intestine was washed with saline at a constant rate of 250 ml/h for one to five hours. A total of 29 hourly specimens were obtained and DNA and protein measured in them. Each specimen was tested for bleeding by Haemocombostix and was negative. Intravenous ^{51}Cr-tagged red cells have previously been shown not to appear in the specimen (Loehry et al, 1969). ^{51}CrCl$_3$-labelled protein loss in three rats washed at 250 ml per hour indicated a loss of 5·1 mg of albumin per hour which compares favourably with the value of 3·6-6·0 mg per hour found by other physiological methods (Katz, Rosenfield, and Sellers, 1961; Wetterfors, Gullberg, Liljedahl, Plantin, Birke, and Olhagen, 1960). Thus at this rate of perfusion there was no evidence that trauma occurred to the mucosa.

In 10 normal rats the rate of washing with saline was altered so that nine specimens were obtained at 150 ml per hour, eight at 250 ml per hour, 10 at 500 ml per hour, and eight at 1,000 ml per hour. There was histological evidence of minor trauma to the mucosa only at the fastest washing rate.

Small bowel washings were performed at a constant rate of 250 ml per hour in 10 rats with high turnover mucosa. High turnover was induced in the mucosa of these rats by infestation with the nematode Nippostrongylus brasiliensis which has been shown to cause a high turnover and loss of intestinal epithelial cells (Symons and Fairbairn, 1962; Loehry et al, 1969). Twenty-seven specimens were analysed for DNA and protein. The results were compared with the 29 specimens from rats with normal small bowel washed at 250 ml per hour.

SUSPENSION OF VIABLE INTESTINAL ABSORPTIVE CELLS

In order to measure the intracellular protein content of intestinal epithelial cells a preparation of isolated epithelial cells was made using pronase to disperse the cells from the mucosa (Harrier, Stern, and Reilly, 1964; Croft and Ingelfinger, 1969). Pronase solution (0·25% in phosphate-buffered saline) was injected into the small intestine lumen of anaesthetized rats and left for 15 minutes at 37°C. After removal the cloudy suspension of cells was filtered through 100 mesh tantalum gauze and washed twice. Examination of the cells by the dye exclusion technique using erythrosin B showed that they were 85-95% viable (Phillips and Terryberry, 1957). Cytology and electron microscopic study confirmed that they were mainly absorptive cells with brush borders (Pink, Croft, and Creamer, 1970) and histology of the mucosa showed that only surface epithelial cells had been removed. The concentration of cells in the preparations was varied by altering the length

of time during which pronase was in contact with mucosa and by varying the degree of agitation of the gut. Fourteen cell suspensions were prepared and analysed for DNA and protein.

SMALL-INTESTINAL WASHINGS IN MAN

Small-bowel washings were performed on six patients at the time of secretin tests of pancreatic function by a method previously described (Croft et al, 1968b). Patients 1 to 4 had no clinical or other evidence of small bowel disease, patient 5 had the coeliac syndrome with a flat jejunal mucosa, and patient 6 had Crohn's disease of the ileum but normal upper small-intestinal biopsy. The method measures cell loss from a 5 cm segment of upper small |intestine without significant contamination by secretions from the stomach, biliary tree, or pancreas. Rates of DNA loss and protein loss were calculated after correction for loss of the unabsorbable marker polyethylene glycol (PEG).

Results

INTESTINAL WASHINGS

The DNA and protein content of intestinal washings from 10 rats with a normal small intestine increased as the rate of washing increased (Fig. 1). There was a highly significant relationship (t = 4·016, P < 0·001) between the loss of these two substances as calculated from the regression line for DNA (axis y) on protein (axis x). (The cause of the increased loss with increasing rates of washing has been shown to be due to improved cleaning of the gut at the faster rates, rather than trauma (P. K. Cotton, personal communication).)
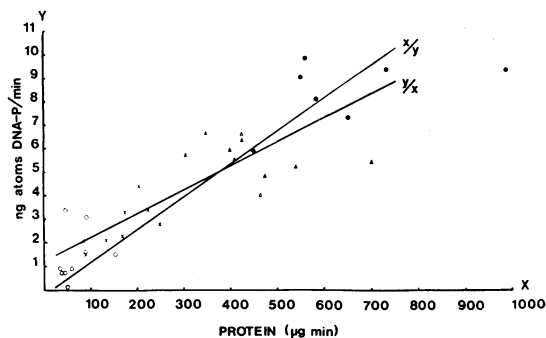

FIG. 1. DNA loss and protein loss from normal rat small bowel washed at different rates. $\bigcirc$ = 150 ml/hour, $\times$ = 250 ml/hour, $\triangle$ = 500 ml/hour, $\bullet$ = 1,000 ml/hour. Two regression lines (x/y and y/x) are plotted. There is a significant relation between DNA (or cell) loss and protein loss (y/x; t = 4·016, P < 0·001).

At a constant washing rate of 250 ml per hour, there was significantly more DNA (P < 0·01) and protein (P < 0·01) in specimens from the infected rats than in those with a normal small-intestinal mucosa (Table I). The ratios of protein/DNA were not significantly different between the two groups.

There was a significant correlation between loss of DNA and protein in the normal rats washed at 250 ml per hour (r = +0·63, P = <0·001). The pooled data for normal and abnormal rats washed

	Intestinal Mucosa	
	Normal (11 rats)	High Turnover (10 rats)
DNA loss rate (ng atoms DNA-P per min)	2·9 SE 0·26 (n = 29)	7·7 SE 0·55[1] (n = 27)
Protein loss rate (μg per min)	159 SE 14 (n = 29)	554 SE 41[1] (n = 32)
Ratio protein loss/ DNA loss	55 (range 23-200)	72 (range 26-138)

Table I *Protein and DNA loss in small-intestinal washings from rats washed at a constant rate of 250 ml per minute*
[1]P < 0·01

at a constant rate are shown in Figure 2. Regression analysis for DNA (axis y) on protein (axis x) proved that this relationship was also significant (t = 3·0, P < 0·01). There was no significant difference between the calculated regression line for DNA loss
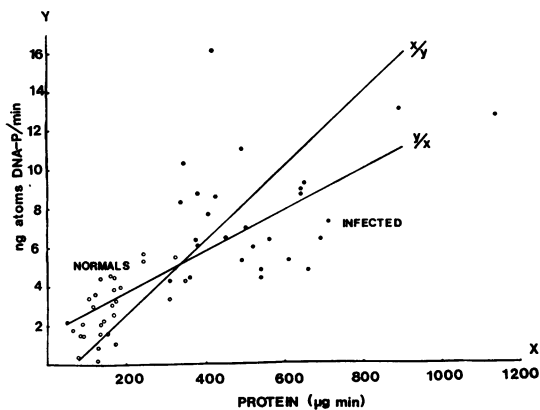

FIG. 2. *Plot of DNA loss (y axis) against protein loss (x axis) for small bowel washed at a constant rate of 250 ml per hour. ○ = normal rats, ● = rats with high turnover mucosa. Regression lines of x/y and y/x are plotted. There is a significant relationship between DNA (or cell) loss and protein loss (y/x: t = 3·0, P < 0·01).*

on protein loss (y/x) for (1) constant rate of washing (250 ml/hr) of normal rats; (2) different rates of washing of normal rats; or (3) constant rate of
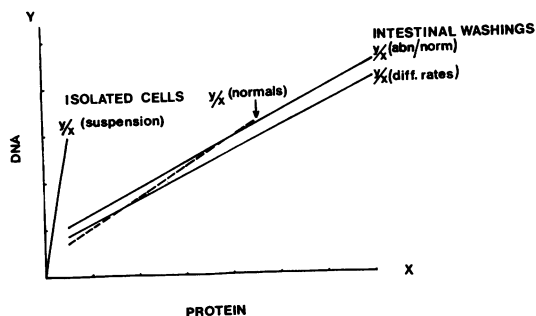

FIG. 3. *Regression lines for DNA against protein of the intestinal washing experiments on right and for isolated rat intestinal cells on left. The broken line is the regression line for rats washed at 250 ml/hour (r = +0·63, P < 0·001). The upper solid line on the right is y/x regression line from Fig. 2 and the lower one is y/x plot from Figure 1. On the left is the regression line on y/x for isolated cells (Fig. 4). There is considerably more protein in relation to DNA in intestinal washings than in isolated epithelial cells.*
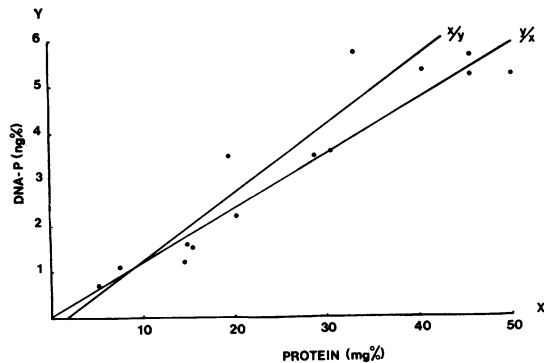

FIG. 4. *DNA and protein concentration of 14 specimens of isolated intestinal cells. Regression lines (x/y and y/x) are plotted. There is a significant correlation between DNA and protein concentration (P < 0·001).*

washing (250 ml/hr) of normal and infected rats (Fig. 3).

ISOLATED INTESTINAL CELLS
There was a highly significant (P < 0·001) relationship between the DNA and protein content of the suspensions of isolated epithelial cells (Fig. 4). The ratio of protein to DNA in the specimen was 8·18

Patient	Age	Sex	Diagnosis	Washing		DNA Loss Rate (ng atoms DNA-P per min)	Protein Loss Rate (µg per min)
				No.	Rate (ml/hr)		
1	52	M	Duodenal ulcer, vagotomy and pyloroplasty	I	18·5	7·8 ⎰ 6·0	1,274 ⎱ 1,030
				II	18·5	4·3	787
2	61	F	Sjögren's syndrome	I	14·7	7·6 ⎰ 6·9	585 ⎱ 371
				II	15·2	6·3	157
3	60	M	Carcinoma pancreas	I	16·1	9·9 ⎰ 11·2	563 ⎱ 521
				II	15·8	12·6	479
4	56	M	Chronic pancreatitis	I	17·8	15·2 ⎰ 17·1	1,221 ⎱ 2,027
				II	16·2	19·0	2,924
5	60	F	Coeliac syndrome	I	12·2	19·9 ⎰ 21·2	182 ⎱ 694
				II	17·2	22·6	1,207
6	60	M	Crohn's disease	I	13·1	36·6 ⎰ 30·7	1,394 ⎱ 1,048
				II	17·8	24·8	803
Mean						15·4	956
SD						9·5	574

Table II   *DNA and protein loss rates from six patients*

(SE = 0·44, n = 14) which was much lower than that for the washings (Table I). This meant that there was considerably less protein in relation to DNA in the isolated cell preparation than in the washings (Fig. 3).

Most of the protein in the washings came from sources other than cells. The ratio of the regression coefficients for the isolated cell and the intestinal washings performed at different rates indicated that only 8·4-14·6% of the protein in the washings was contributed by intracellular protein. When the calculation was performed using the pooled data from normal and abnormal rats washed at a constant rate, the percentage of protein contributed by cells was calculated to be 9·4-11·1%, which was within the same range.

HUMAN DATA
The results of the observations in man are recorded in Table II.

Discussion

Using two experimental models in rats a highly significant relationship was found between the loss of total protein from small intestinal mucosa and the loss of DNA (or cells). Only 8-15% of the protein in intestinal washings arose from intracellular protein; 85-92% was extracellular protein. The extracellular protein could have come from a number of sources, such as plasma, mucoproteins, and interstitial fluid. One interpretation of the data is that extracellular protein is lost with the epithelial cell as it exfoliates, perhaps through the hole left by the extruded cell. If so, cell loss would explain

protein-losing enteropathy in the active coeliac syndrome, for in this condition there is a higher than normal loss of epithelial cells (Croft, Loehry, and Creamer, 1968a).

Our data for protein loss from the small bowel mucosa of man is derived from six subjects (Table II). The small bowel DNA-loss rates of patients 1-4 were within the normal range for patients with normal small-intestinal mucosa (Croft *et al*, 1968b). None of these patients had evidence of mucosal disease of the small intestine. Two patients (5 and 6) had small bowel disease and the rates of DNA loss of both were above the normal range. Patient 5 had the coeliac syndrome and was losing weight, a clinical situation previously reported to be associated with high DNA loss (Croft *et al*, 1968a). The other patient had Crohn's disease involving the terminal small bowel. The mean small bowel DNA-loss rates of the six patients was 15·4 (SD = 9·5) ng atoms DNA-P per min which was within the normal range. The ratio of protein to DNA loss was 62·1, a value in the same range as that found in the rat intestinal washings (Table I). The mean protein loss for the six subjects was 956 (SD = 574) µg per minute. If one assumes that this rate was constant throughout the 24 hours, our measured value represents a loss from 5 cm of jejunum of 1·4 g protein per 24 hours.

Because of the small number of studies we do not claim that the human data reflect the physiological situation, but we do believe that it gives a figure for the order of magnitude of protein loss from human small bowel mucosa. Our value of 1·4 g per 24 hours was the loss from only 5 cm of small intestine. The small bowel is some three hundred centimetres long

and, if protein loss occurs at the same rate throughout its length, 84 g would be lost per 24 hours, about 10 g arising from intracellular protein of exfoliated cells. These figures may be overestimated, as protein loss is likely to be less in the ileum but they are of the same order as, or less than, the values discussed by others (Spencer, 1960; Wilson, 1962; Nasset, 1964 and 1965). We conclude that the amount of protein lost from human small bowel mucosa per 24 hours is substantial and that it is related to the rate of loss of epithelial cells. Presumably the normal intestine digests and absorbs this protein. Irretrievable loss of protein from the body will occur when the intestine is diseased, has a high rate of cell and protein loss, and is also less able to reabsorb, as in the coeliac syndrome.

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