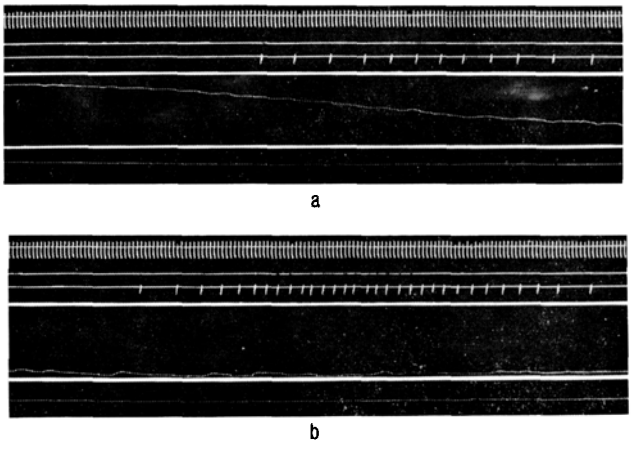
The pupils were inspected at intervals. In 2 of the cats the denervated pupil was found to be considerably larger than the normal one while the membrane was contracted, and even, to a smaller extent, after its relaxation. Degeneration contraction of the pupillary dilator, difficult to find in unanaesthetized cats[8], can thus occasionally occur.

Unprovoked degeneration secretion from the sympathetically denervated submaxillary gland was found in 4 cats. It appeared later than the contraction of the membrane, while the membrane was relaxing or later, even at the end of the experiments 38–40 h after ganglionectomy. The rate of flow varied and between periods of activity there were intervals of secretory rest, as shown in the Figure and described earlier[7]. In 4 other cats degeneration secretion could be provoked 32–37 h after ganglionectomy by injecting noradrenaline i.v. After the immediate secretory response to this drug, there was a pause of 5–7 min, and secretion was then recommenced. In 2 cats there was no degeneration secretion.

The Figure shows degeneration secretion appearing $26^1/_2$ h after ganglionectomy, when the degeneration contraction of the membrane was wearing off. A second, and more marked period of secretion 30 h after ganglionectomy is also shown. At that stage the membrane had relaxed fully. It is evident that contraction and secretion occur independently of each other; they cannot be due to catecholamines in the blood but must be caused by local events. On the whole, secretion appears later than the contraction. This may reflect some difference between the secretory fibres of the gland and the motor fibres of the membrane; it seems questionable whether an explanation can be provided by the finding[10] that the time of appearance of degeneration activity depends on the length of the degenerating nerve. That degeneration secretion was obtained less regularly than contraction of the membrane is very likely due to the fact that the sympathetic secretory innervation of the cat's submaxillary gland is variable, being sometimes extensive, sometimes very scanty[11,12].



Degeneration contraction and degeneration secretion. (a) Started $25^1/_2$ h, (b) $29^1/_2$ h after sympathectomy. Records in each section from above: time in min, secretion from the left, from the right submaxillary gland and contractions of the right and the left nictitating membrane.

Zusammenfassung. Nach Sympathectomie erscheint eine «Degenerationskontraktion» der Nickhaut nicht nur in wachen[8] oder spinalen[9], sondern auch regelmässig in narkotisierten Katzen. Die «Degenerationssekretion» von der Submaxillarisdrüse tritt später auf; aber auf Grund der Variabilität der sympathisch sekretorischen Innervation sieht man sie nicht bei allen Tieren.

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Institute of Physiology, University of Lund (Sweden), 5 September 1967.

[10] N. Emmelin, J. Physiol. *188*, 44P (1967).

[11] N. Emmelin, in *Handbook of Physiology, Alimentary Canal I* (Waverly Press Inc., Baltimore 1967).

[12] Supported by a grant from the Swedish Medical Research Council.

A Histoautoradiographic Study of Protein Loss in the Intestine of Antifolic-Treated Rats

Since 1962 in our laboratory we have been able to work up a model of enteropathy in rats treated with different dosages of antifolic drugs or antibiotics. More recently, histological changes of intestinal mucosa and metabolism of Cr^{51}-albumin have been investigated in these animals (Marano et al.[1]). Our experiments showed albumin losses from the bowel and a parallel fall of protein plasma levels.

The purpose of this study is to clarify the mechanism of protein loss and to establish a relationship between experimental and human pathology.

One hundred male albino rats (weighing between 200 and 250 g) were divided into 3 groups: the first group of 40 rats received by stomach tube 0.2 mg/kg body weight of aminopterine daily for 7 days; the second group of 40 rats was given the same drug at the dosage of 0.04 mg/kg daily for 60 days; the rats of the third group, used as a control, received 2 ml of saline daily.

At the end of the treatment, each rat was injected with 15 μg of Cr^{51}-albumin into the vein of the tail. The rats were sacrificed in groups of 7, respectively 6, 12, 24, 48 and 72 h following injection of albumin, and a loop of the small bowel was taken for histology. This sample was washed in saline and fixed in 10% formol saline. The presence of labelled albumin in the intestinal mucosa was demonstrated by means of the autoradiographic technique; radioactive Cr in the sections appeared as black spots.

Autoradiographic technique. After fixation, the specimens were washed in water, dehydrated and embedded in paraffin at 56–58 °C.

The sections, 5–7 μ thick, were placed in water at 37 °C, distended on clean slides and kept overnight at 37 °C; the paraffin was then removed and the sections were left for 5 min in absolute alcohol to dry at room temperature.

The procedure was carried out in a dark room (Wratten Safelight No. 1 lamp) as follows. Rectangular pieces of film (Kodak autoradiographic plates type A) as large as

[1] R. Marano, G. Pastore and O. Schiraldi, Rass. Fisiopat. clin. terap. *37*, 544 (1965).

the slides were laid face downwards on the surface of deionized water at 20 °C; the slide was then immersed under the film so that, moving it upwards, the film stuck into it. After securing adhesion between slide and film by drying, the sections were placed in a film box and this was sealed off.

After 30 days, the films were developed (Kodak D solution) and fixed (Na hyposulphide).

Results. (1) In the specimens of animals sacrificed 12 and 24 h after injection of albumin, radioactive material was observed only in the capillary vessels, muscularis mucosa and chorion. (2) The specimens of animals sacrificed 48 and 72 h after injection of albumin show nearly all the radioactive material in immediate proximity to epithelial cells lining the mucous glands. (3) The bowel of rats treated for 7 days showed a high degree of radioactivity; this phenomenon was less evident in the group treated for 60 days.

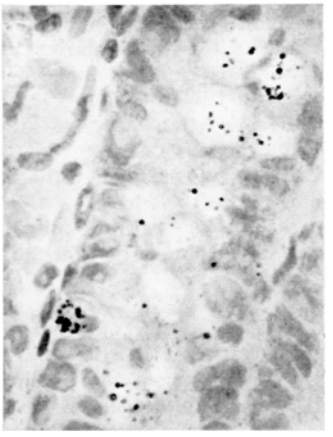

Fig. 1

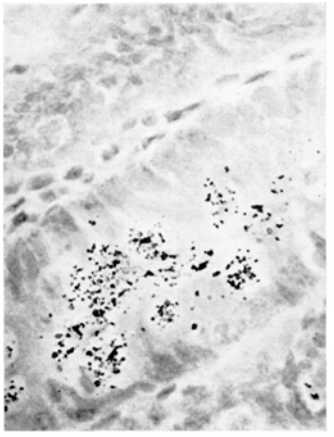

Fig. 2

Fig. 1 and 2. Small bowel of rat treated for 60 days and sacrificed 48 h following injection of Cr^{51} Albumin. Many mucous cells show in the distal part of their cytoplasm a large and clear vacuole which contains radioactive material.

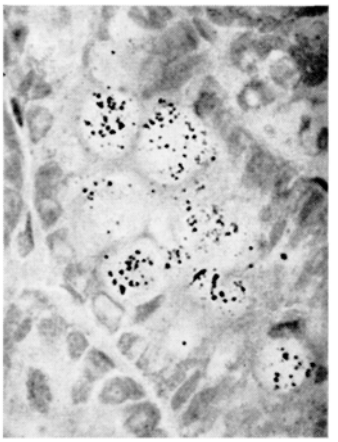

Fig. 3. A mucous cell during the secretion phase shows diffusion of Cr^{51} Albumin into the intestinal lumen.

These autoradiographic observations confirm the results of previous experiments in which blood level and fecal excretion of Cr^{51}-albumin were determined (MARANO et al.[1]). The relationship between severity of histological lesion and albumin loss has been confirmed as well.

We shall discuss here in detail only the histological pattern of specimens taken 48 h following injection of albumin in rats treated for 60 days. This we do for 2 reasons. The first is that the histological changes in the mucosa of the animals of this group were less marked than those of the other groups, so that the cellular structure could be demonstrated to a better extent; the second is that the functional and anatomical lesions in these rats seem to be very similar to those seen in human pathology and particularly in sprue (SCHIRALDI and MARANO[2]; MARANO[3]).

In fact, the lesions due to chronic treatment with aminopterine are not caused by inhibition of mitosis; on the contrary there seems to be an increasing mitosis rate and a rapid epithelial proliferation. The villi are shorter and slightly thicker but well apart from one another and lined by young epithelial cells at times not fully differentiated, particularly at the base of the villus.

The number of mucous cells appears to be increased. These elements show generally picnotic nucleus and granular hyper-eosinophilic cytoplasm. They show also frequently in the distal part of their cytoplasm a large and clear vacuole which contains radioactive material. The black spots are usually scattered, but some of them gather in larger blocks. A few mucous cells during the secretion phase show diffusion of Cr^{51}-albumin into the intestinal lumen. In some slides this is shown by the presence of black spots in the basal membrane of epithelial cells.

The data we have briefly summarized are in full agreement with the observations of others (HOLLANDER et al.[4]).

A close similarity between mucous secretion and intestinal protein loss is the main finding of this study. In our experimental conditions, protein loss would seem to occur mainly through the mucous cells, even though severe mucosal damage did not appear.

Riassunto. È stata osservata con istoautoradiografia la modalità del passaggio della albumina nell'intestino di ratto trattato con aminopterina. Si è potuto dimostrare che la perdita proteica nel lume intestinale avviene tramite le cellule mucipare per un processo di pinocitosi.

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C. STRADA and R. MARANO

Department of Medicine, University of Bari (Italy), 3 August 1967.

[2] O. SCHIRALDI and R. MARANO, Progresso med., Napoli *18*, 495 (1962).
[3] R. MARANO, Boll. Soc. ital. biol. Sper. *39*, 599 (1963).
[4] F. HOLLANDER and M. I. HOROWITZ, Gastroenterology *43*, 75 (1962).