Relative and Absolute Goblet Cell Numbers in Intestinal Crypts Following Irradiation or Actinomycin D

Mammalian intestinal crypts can be subdivided into proliferative and differentiated compartments (goblet cells, maturing columnar cells, Paneth cells, argentaffin cells). The largest groups of non-dividing crypt cells are goblet cells and maturing columnar cells. Following irradiation, the intestinal mucosa attempts to compensate for damage by expanding the proliferative compartment and shortening the cell cycle[1,2]. The proliferative compartment expands at the expense of the maturing columnar cell compartment, which virtually disappears. Several investigators have reported changes in goblet cell numbers (both increases and decreases) during this post-irradiation period[3-6]. In order to further investigate this phenomenon, a study was undertaken utilizing X-radiation and actinomycin D as cytotoxic agents to alter the crypt cell populations.

Materials and methods. Male C57 Bl/J mice, 100–120 days old, were used in both experiments. In the first experiment 25 mice were irradiated, abdomen only, with 600 R of X-radiation (HVL 1.8 mm copper). Groups of 5 mice were sacrificed by cervical dislocation at 8 through 96 h post-irradiation, and samples taken from the lower ileum. The samples were fixed in cold (4 °C) ethanol: acetic acid (3:1), stained by the Feulgen reaction, crypts dissected free, and crypt squashes prepared[7]. Goblet cells were stained with alcian blue, 0.1%, pH 2.5.

In the second experiment, 25 mice were injected i.p. with actinomycin D (Cosmegen®, Merck, Sharp and Dohme), 750 µg/kg body weight, and crypt squash preparations made as before. Total cells and total goblet cells per crypt were determined for 20 crypts per animal for both experiments. The relative number of goblet cells for each animal was determined by dividing goblet cells/crypt by total cells/crypt (expressed as percent).

Results and discussion. Following 600 of X-radiation, total cells per crypt were decreased for the first 48 h post-exposure, and by 96 h the crypt was in a hyperplastic state (Figure 1). Total goblet cells also decreased for the same period, but not as drastically as total crypt cells (Figure 2). The result was an increase in the percentage of crypt cells which were goblet cells (Figure 3). A similar pattern was seen following administration of actinomycin D, but recovery was delayed about 1 day in comparison with X-radiation. The maximum goblet cell percentage therefore occurred 1 day after the peak with X-radiation (Figure 3).

This phenomenon can best be explained by differences in sensitivity between crypt cell populations. Proliferative

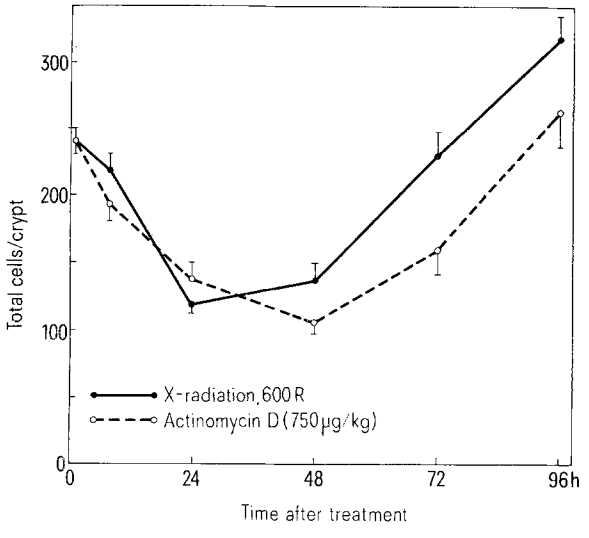

Fig. 1. Total cells per ileal crypt following X-radiation or actinomycin D administration. Mean ± 1 standard error.

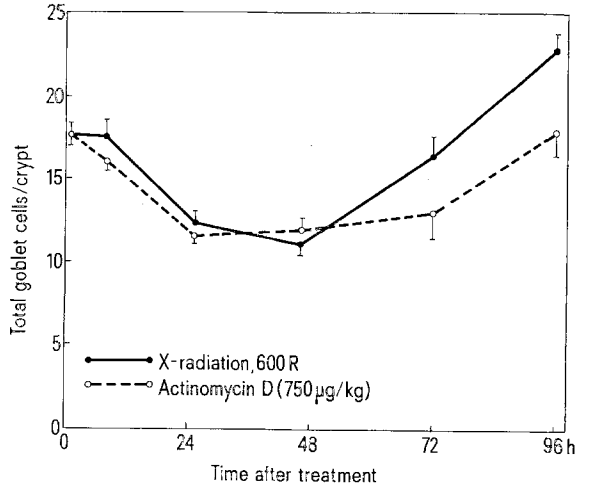

Fig. 2. Total goblet cells perileal crypt following X-radiation or actinomycin D administration. Mean ± 1 standard error.

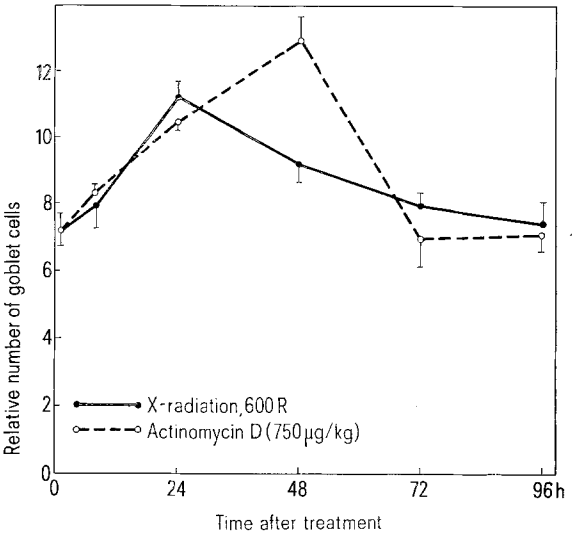

Fig. 3. Relative number of goblet cells in ileal crypts following X-radiation or actinomycin D administration. (Goblet cells/crypt)/(Total cells/crypt), in percent. Mean ± 1 standard error.

[1] S. Lesher, Radiation Res. *32*, 510 (1967).
[2] S. Lesher and J. Lesher, Radiation Res. *43*, 429 (1970).
[3] N. Friedman, J. exp. Med. *81*, 553 (1945).
[4] S. Lesher, J. natn. Cancer Inst. *19*, 1949 (1957).
[5] G. Wiernik, Br. J. Radiol. *44*, 348 (1971).
[6] W. Montagna and J. Wilson, J. natn. Cancer Inst. *15*, 1703 (1955).
[7] D. E. Wimber, H. Quastler, O. L. Stein and D. R. Wimber J. biophys. biochem. Cytol. *8*, 327 (1960).

cells are in cycle and thus are sensitive to cytotoxic agents; goblet cells are not in cycle and thus are resistant. Their decrease in absolute number is probably due to killing of their proliferative precursors, coupled with continued migration out of the crypt.

Differences in sensitivity may also explain WIERNIK's[5] data, which describes a wave of goblet cells migrating out of the crypt following irradiation. Since goblet cells and proliferative cells are normally intermingled in the crypt, an agent which kills a portion of the intervening proliferative cells, creates a group of goblet cells which may then migrate together as a wave.

All previous work on goblet cell kinetics has been done with tissue sections, which may give a misleading impression of dramatically increased goblet cell number because of an increase in the size of these cells following irradiation. Most investigators have been correct in reporting a goblet cell increase, but have failed to distinguish between absolute and relative numbers.

Résumé. Les modifications apparaissant dans les cellules caliciformes de l'iléum des souris après irradiation ou l'administration d'actinomycine D sont en premier lieu la répercussion des changements survenus dans la zone de prolifération.

J.W. COOPER[8,9]

Cell and Radiation Biology Laboratories, Department of Radiology, Allegheny General Hospital, 320 E. North Avenue, Pittsburgh (Pennsylvania 15212, USA), 27 November 1973.

[8] This work was supported by N.I.H. Grants No. 1 PO2 CA 104438-06 and 5 TO1 CA05224-04.

[9] Technical Assistance was provided by Mrs. JEANNE KISSEL and Mrs. LYN COST.

Effect of Hyperventilation on the Platelet Aggregation Induced by ADP

It was found previously[1] that circulating platelets show a higher responsiveness to ADP before their passage through the pulmonary vessels than after it; in fact, the platelets of the arterial blood show a lesser extent in the maximal aggregation compared to the platelets of the venous blood. This different behaviour is related to a plasmatic component released or metabolized during the passage of blood through the pulmonary vessels[2]. This result gives an account of the interaction between the lungs and the platelet's functions also observed by other authors[3-8]. Additional evidence of these connections is provided by the effect of an artificially produced hyperventilation on the aggregative behaviour of the platelets, which is reported in this paper.

Methods. Adult rats of both sexes were anaesthetized with ether and then with urethan (Carlo Erba, Milano, 400 mg/kg body wt.) and Na Nembutal (Abbot, Aprilia, 30 mg/kg body wt.)

Ventilation was performed by inflating the lungs with air by means of a small-animal respiratory pump (Scientific and Research Instruments LTD, Croydon, Surrey) connected to a cannula inserted in the trachea. The volume/min was adjusted to 0.6 l/min (85 breaths/min for 5 min), in rats hyperventilated and to 0.3 l/min (35 breaths/min for 5 min) in the controls, a value which agrees with normal quiet respiration. Then the chest of the animals was opened and the arterial blood was collected by cardiac puncture from the left ventricle. Sodium citrate (3.8% mixed 1:9) was used as the anticoagulant.

The preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) and also platelet count were carried out as previously described[1].

The platelet aggregation was studied by means of an aggregometer (169 Platelet Aggregation Meter-Evans Electroselenium Ltd) to measure the changes in the optical density (O.D.) of PRP during aggregation induced by ADP (Na_3ADP – C.F. Boehringer and Söhne H-Mannheim – final concentration 9.2×10^{-6} *M*).

In two groups of experiments, sodium lactate (The British Drug Houses Ltd – B.D.H. Laboratory Chemical Division – final concentration 20 mEq/l) was added to blood just before the PRP preparation, or to PRP and incubated for 3 min at 37 °C in the aggregometer before adding ADP.

The aggregation curve was measured in the following way: the maximum curve height was measured from the baseline to the midpoint of the highest segment of the curve, to estimate the extent of aggregation. Statistical analysis was performed with the 2 sample *t*-test for the limiting value of 0.05 probability[9].

Results. Platelets of hyperventilated animals show a greater degree of responsiveness to ADP in comparison with platelets of normally ventilated animals. The values obtained in the experiments are reported in the Table; the mean value of the maximal extent of aggregation was 49.72% (S.D. ± 4.62) in the hyperventilated rats

Maximal extent of platelet aggregation by ADP in PRP obtained from hyperventilated rats (8 experiments) and from normally ventilated rats (8 experiments)

Hyperventilation	Control
51.40	34.80
49.04	30.80
50.00	41.60
49.20	40.40
46.20	36.00
41.32	41.00
55.40	39.20
55.32	40.92
Mean 49.72 (S.D. ± 4.62)	Mean 39.21 (S.D. ± 2.48)

$t = 5.664 > 2.120$ for P 0.05.

[1] D. BOTTECCHIA and M. G. DONI, Experientia *29*, 211 (1973).
[2] D. BOTTECCHIA and M. G. DONI, Experientia *29*, 341 (1973).
[3] R. H. ASTER, J. H. JANDL, J. clin. Invest. *32*, 954 (1964).
[4] V. M. ZYABLITSKII, Bull. exp. Biol. Med. *68*, 1073 (1969).
[5] K. U. BENNER and K. E. FREDE, Pflügers Arch. *328*, 185 (1971).
[6] S. E. BERGENTZ, D. H. LEWIS and V. LJUNGQUIST, in Sixth Europ. Conference on Microcirculation (Karger, Basel 1971), p. 35.
[7] U. LJUNGQUIST, in *Platelet Aggregation* (Ed. J. CAEN; Masson & Cie, Paris 1971), p. 227.
[8] M. KIEN, H. HECHTMAN and D. SHEPRO, Microvasc. Res. *3*, 209 (1971).
[9] S. KOLLER, *Metodi Statistici Generali per il Biologo* (Editoriale, Milano 1947), p. 102.