propriate circumstances. None of these tests however give any indication of the state of the reovirus 3 antigen. Standard methods for isolation and visualization of virus have proved, with one exception, unsuccessful (10, 6). While these methods may not be sensitive enough to detect low concentrations of reovirus 3, experiments with fluorescent antibody and the enhancement of infectivity by proteolytic enzymes (14) which are in progress may produce information regarding this very important point.

In the light of the postulated viral etiology for Burkitt's lymphoma (15), and the results reported here, we suggest that consideration of the existence of viral information in a complex haptenic form should be considered not only in the case of Burkitt's lymphoma, but in any general concepts of viral etiology for neoplasia. Techniques designed specifically to detect such complex haptens should therefore be routinely applied in the search for viral information contained in neoplastic cells.

Summary. Cell fractionation studies on 2731/L lymphoma cells and the use of these for the production of antisera have shown that a reovirus 3 "antigen" present initially may have undergone a transition into a complex haptenic form during the course of successive transplantation passages. The complex hapten is fully antigenic for the rabbit and using this property, it has been shown to reside in light mitochondrial and microsomal fractions of the 2731/L lymphoma. It is suggested that the hapten is in fact associated with the polyribosomes/microsomes of the lymphoma. The results are discussed with respect to the viral etiology of Burkitt's lymphoma.

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Effect of Exposure Conditions on the Production of Radiation Lesions in Rat Small Intestine (32842)

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Although most investigators (1) have described the morphologic response following irradiation of the small intestine as a uniform generalized phenomenon, there are occasional contradicting reports of uneven morphologic changes even in the same species irradiated

under similar conditions (2–4). The reasons for these discrepancies are not clear. The present experiments were designed to investigate the morphologic characteristics of lesions in the small intestine of the rat under a variety of conditions and to correlate the differences in response with different conditions of radiation exposure.

Materials and Methods. Female Wistar rats (180–200 gm) housed individually in wire bottomed cages, fed a standard diet[1] and allowed water *ad libitum,* were used in all experiments. The rats were anesthetized with pentobarbital (5 mg/100 gm of body wt.) prior to all surgical procedures or radiation exposures and necropsied following an overdose of pentobarbital. Control rats, unless otherwise specified, were subjected to the same procedures, but were not irradiated. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Irradiation procedures. Rats were exposed to an air dose of 2000 R at 143 R/min, as determined by a Victoreen R meter, delivered by a 250 kVp X-ray unit with a 0.5 mm copper and 1 mm aluminum filter. Shielding was provided by a 0.25-inch thick sheet of lead. The small intestine was irradiated by the methods listed below with the number of rats in each experiment in parentheses:

I. Whole body irradiation (4 irradiated and 3 control) with the rats positioned on their sides (Fig. 2, Group I).

II. *In situ* irradiation (6 irradiated and 3 control) with the rats placed in the supine position, the abdomen exposed and the upper body and the lower limbs shielded (Fig. 2, Group II).

III. Irradiation of the exteriorized intestines (36 irradiated and 28 control in 5 separate experiments). The small intestine, the cecum, and part of the colon were gently teased out through a 5-cm midline incision and a small forked holder was placed under the root of the mesentery to prevent spontaneous retraction. Rats were positioned on their side under shields so that only the exteriorized gut, covered with gauze saturated with warmed 0.9% saline, was irradiated. After irradiation, the bowel was reinserted and the incision closed with Michelle clips (Fig. 2, Group III). As it is known that hypoxia may decrease the severity of radiation (5, 6), alterations of the regional intestinal blood flow were produced in groups of rats as follows: (a) The mesenteric artery was ligated (6) (5 ligated and 3 control, sham ligated and irradiated), (b) Phenoxybenzamine hydrochloride,[2] a long acting adrenergic blocking agent, was injected intramuscularly (4 mg/kg of body wt.) a few minutes prior to the exteriorization of the gut and irradiation (7 irradiated, 2 control) (Fig. 2, IIIa. and b.). Attempts to protect the terminal ileum either by ligating the blood vessels leading to it or by treating them externally with Levarterenol bitartrate[3] produced inconsistent results and will not be reported.

Morphologic procedures. In one of the Group III experiments, rats were sacrificed at daily intervals for 5 days after irradiation. All other rats were sacrificed on the third day or later, when the anatomic lesions were most severe. The small intestines were excised, opened immediately, and fixed pinned flat in 10% buffered formalin. The entire length of the small intestine was examined under a dissecting microscope (×25) and the distribution of lesions was mapped. Gross observations were verified microscopically on routinely prepared paraffin sections from representative areas. Short segments of jejunum and ileum were fresh-frozen for the preparation of cryostat sections which were stained for fat with oil red O (ORO).

The length of the small intestine was measured either before or after fixation. To preclude the effect of differential stretching during these manipulations, the abdominal viscera of 10 irradiated and 13 control rats were removed and fixed *en bloc;* only following fixation were the small intestines dissected free and measured.

Observations and Comment. At necropsy the small intestine, irrespective of the method

[1] Rat and Mouse Baked Biscuits, D and G Laboratory Animal Food, Frederick, Md.

[2] Dibenzyline HCl, Smith, Kline and French Laboratories, Philadelphia, Penna.

[3] Levophed, Winthrop Laboratories, New York, N. Y.

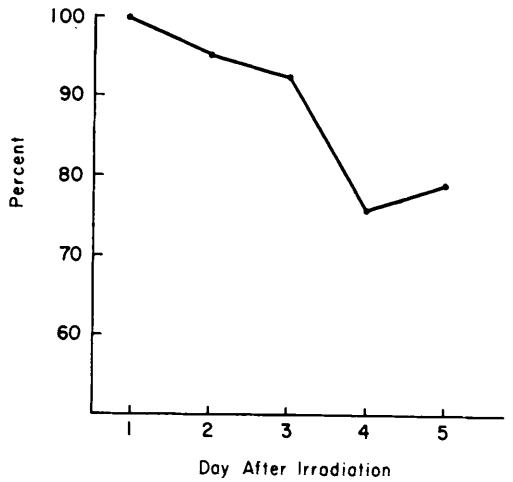

FIG. 1. Measurements (mean length) taken on unfixed opened small intestines from one series of rats of exposure group III; expressed as a percentage of the mean length of sham-irradiated control small intestine.

of radiation exposure, was found to be congested, edematous, and filled with mucinous fluid. Blood was often present in the lumen of rats that were moribund at the time of sacrifice. After the first day the irradiated guts were found to be progressively shorter than the nonirradiated guts (Fig. 1). The mean length of the irradiated guts fixed without manipulation was two thirds of the mean length of the control guts, i.e., 40.5 cm and 62.8 cm, respectively. If these lengths are compared with those of intestines fixed opened (Fig. 2), it is apparent that pinning the bowel flat before fixing prevents some shrinkage but does not alter the difference in length between the 2 groups. The etiology of the shortening is not apparent. Burn and his collaborators (7) have demonstrated a loss of acetyl cholinesterase in the bowel following irradiation, with a possible increase in the contractility of the muscular layers. In our sections, however, the muscle coat does not appear contracted; the shortening of the gut could be related to the marked thickening of the smooth muscle fibers, due to edema similar to that described in cases of fatal human radiation accidents (8).

The mucosa of the small intestine of both the whole-body and the *in situ* gut irradiated rats developed the uniform classic lesions of the acute intestinal syndrome (1). We did not confirm the previous reports (2, 4) of an earlier and more severe involvement of the duodenum. Our results are also at variance with those of Sullivan and his associates (3) who reported an uneven intestinal response in a similar *in situ* experiment. Their observation could have been due to the small size of the window in the shield which allowed exposure of only limited portions of the gut to the dose.

In contrast to the uniform reaction of the whole-body and *in situ* irradiated gut we found that the reaction of the exteriorized small intestine to ionizing radiation was uneven and that the pattern of injury was not consistent (Fig. 2). During the first 2 days after irradiation, when the damage was most evident in the crypt epithelium, areas with severely affected crypts alternated with nearly normal ones. In the following days the contrast between nearly normal and severely injured areas became more pronounced (Figs. 2 and 3) and could be distinguished grossly. The characteristic foamy appearance of the epithelial cells covering the irradiated mucosal surface after the third day was due to the presence of large lipid droplets (Fig. 3D). Similar lipid droplets have been observed in the vacuolated epithelial cells of irradiated dog gut (personal observation). In electron microscopic studies of irradiated rat and mouse intestines, lipids have been reported in epithelial cells (9–11), but they have not been related to the vacuolization seen in light microscopy. The size and distribution of the lipid droplets argues against an alimentary origin. Their presence has been considered evidence of cellular injury mediated through interference with protein and lipoprotein synthesis which results in fat accumulation (11).

On days 4 and 5 after irradiation scattered hemorrhagic and ulcerated areas were observed. Elsewhere the epithelial covering was remarkably continuous, but could easily become detached through mishandling. At no time was it possible to find the generalized denudation of the mucosa mentioned in the literature (1, 8).

The results of the experiments conducted to test the effects of alterations of blood flow on the response of the small intestine to irradiation are illustrated in Fig. 2. A notable

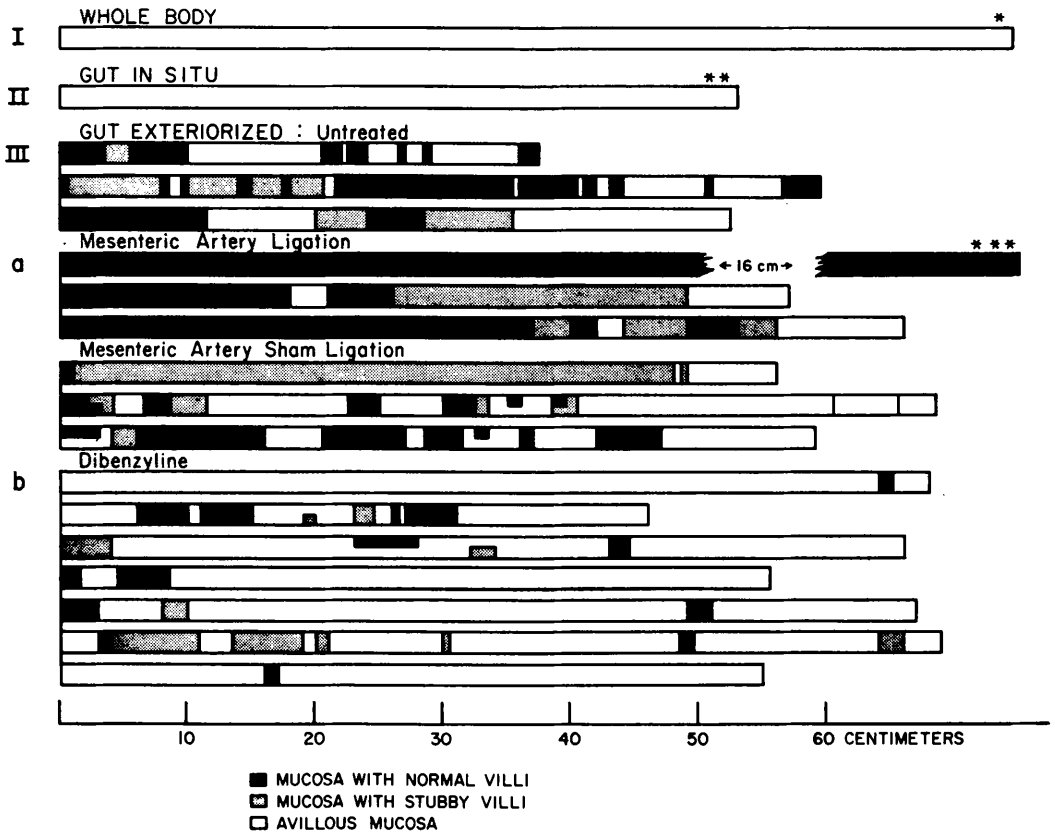

FIG. 2. Pattern of response of the rats small gut to various methods of X-irradiation. The bars represent the whole length of the small gut from the pylorus on the left to the ileocecal valve on the right. *Mean length of the small gut from 4 rats, 3 days after irradiation; **from 6 rats, 4 days after irradiation—notice the progressive shortening of the gut; ***from 3 rats, 4 days after irradiation—as the mucosa is undamaged so is the gut longer. Each bar unmarked by asterisks represents the small gut of one rat.

degree of protection against irradiation was associated with temporary ligation of the mesenteric artery (Group III, a), as previously shown (6). Conversely, treatment with Dibenzyline (Group III, b) appeared to result in a more uniformly severe reaction, presumably by preventing vascular constriction. The small intestines of all sham irradiated rats showed no lesions.

We want to alert investigators to this variability of the response of the exteriorized small intestine under apparently similar experimental conditions, since it could well affect experiments using this model. We want also to underline the danger of evaluating pathologic changes in the intestinal mucosa on the basis of limited samples only, without at least a gross examination of the whole mucosal surface.

Conclusions. The 2000 R X-irradiation of small gut of the rat in general produces a shortening of the viscus which is time dependent. This dose, when delivered either to the whole-body or to the abdomen only, produces in the small gut of the rat the uniform classic lesion of the acute intestinal radiation syndrome, but without generalized denudation. The characteristic, foamy, epithelial cells that line most of the luminal surface after the third day are packed with large lipid droplets. The same dose, when applied to the exteriorized gut produces patchy lesions with areas similar to the above, interspersed with apparently intact areas usually concentrated in the upper

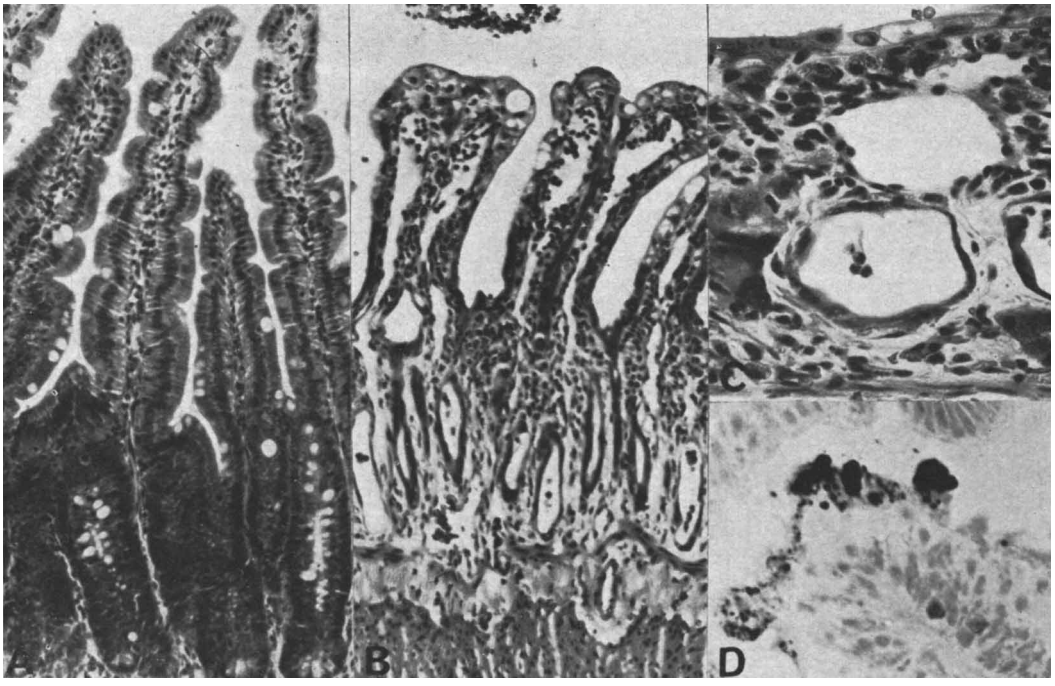

FIG. 3. Exteriorized gut, 3 days after irradiation. H and E. (A) Mucosa from nondamaged areas with well developed villi and normal epithelium; × 180. (B) Mucosa with radiation damage from area with "stubby" villi. The villous structure is still evident; notice the foamy, misshapen epithelial cells of the villi and the flattened epithelium of the cystic crypts; the lacteals are markedly dilated; × 160. (C) Avillous mucosa from area of severe radiation damage. Although the villous structure has completely disappeared, the flattened epithelial lining is continuous; × 330. (D) Mucosa with "stubby villi": Fat stain reveals irregular fat droplets filling the vacuoles of the foamy cells, ORO; × 330.

small intestine. There are indications that the intact areas might have escaped the full effect of the dose delivered due to some interference with their blood supply resulting from the process of exteriorization.

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