the tidal flow setting up an unstable condition, which is followed by mixing of the whole water column during flood and high water, aids in the movement of salt against the mean flow of the river passing through the estuary. This occurs at all stations from the mouth to the head at all times of the year. The data were insufficient to show whether the density inversions were more likely to occur at any particular stage of the river.

In addition to assisting in the maintenance of the salt balance in the estuary, the tidal overmixing causes ventilation of the bottom waters at frequent intervals. Oxygen, nutrients, plankton, pollutants, and any other suspended or dissolved materials are mixed throughout the whole water column on almost every tidal cycle throughout the year.

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2. This work was supported in part by an Office of Naval Research contract and by Oregon State College.
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X-ray Induced Chromosome Aberrations in Normal Diploid Human Tissue Cultures

In recent years, and particularly in recent months, the estimation of radiation hazards to human populations has become very important. While there is an enormous body of data on other organisms, there are few data on human beings. Without some indication of the sensitivity of humans in relation to the better known organisms, it is difficult to make quantitative estimates of radiation hazards to humans.

The recent development of simple, reliable methods of tissue culture, especially those developed for virus research (*1*), make it easy to grow mammalian cells for genetic and cytological studies. The discovery by Hsu and Pomerat (*2*) of an improved method of preparation of tissue-culture cells offered the possibility of making cytological studies of radiation damage to human chromosomes by direct examination for aberrations, as is done with the classical plant materials.

Such a study must, of course, be made on diploid cells derived from normal tissues. Many existing human tissue-culture cell lines derived from normal tissue have been examined cytologically by myself and other workers; but, unfortunately, they have all turned out to be polyploid, apparently basically tetraploid, with wide aneuploid variations. Since it was known from work on monkey kidney chromosomes in our laboratory that a newly set line did not become basically polyploid until after at least the sixth passage, a new line was set from normal human kidney. The newly cultured tissue was found to have 46 chromosomes and, from the first to the fourth passage, to contain an average of only 8 percent of polyploid cells.

Table 1. Aberrations in cultured epithelioid diploid human kidney cells after treatment with x-rays.

Time (hr)	Dose (r)	Cells scored (No.)	Chromatid deletions (No.)	Isochromatid deletions (No.)	Chromatid exchanges (No.)	Total breaks (No.)	Breaks per 100 cells (No.)
42	Control	150	1	0	0	1	0.7
42	25 r	147	2	6	1	10	6.8
42	50 r	74	3	11	1	16	21.6
49	Control	150	1	1	0	2	1.3
49	25 r	150	2	6	0	8	5.3
49	50 r	133	2	13	2	19	14.3
72	Control	67	0	0	0	0	0.0
72	25 r	60	0	2	0	2	3.3
72	50 r	43	0	2	0	2	4.7

For the present studies (*3*), epithelioid cells for the second to the fourth serial cultures were used. The cultures were made from a normal kidney which was removed from a 1-year-old female patient at the Johns Hopkins Hospital (*4*). The kidney cultures were prepared by a modified Younger (*1*) technique and grown in a modified Chang's medium (*5*). The experimental cultures were made on cover slips in Leighton tubes. When a good sheet of cells had grown on the cover slips (3 to 6 days), the tubes were used for irradiations.

The x-irradiations (*6*) were performed with a G.E. Maxitron therapy machine. It was operated at 250 kv (peak) and 15 ma with filtration through 1 mm of aluminum and 1 mm of copper. The half-value layer was 2 mm of copper. Two doses, 25 and 50 r, were used.

The nutrient solution was drained from the tubes before they were treated and was replaced with fresh solution afterward. Control tubes were handled in exactly the same manner in this and all other respects, excepting actual irradiation. About 15 hours before the cultures were to be fixed, colchicine was added to a final concentration of 10^{-7} *M*. The cells were first incubated for 20 minutes in a 20 percent BSS solution and then fixed in Darlington and LaCour's 2BD and stained by the Feulgen method.

Preliminary experiments had shown that the most favorable time interval between irradiation and fixation was 40 to 72 hours. Aberration counts were made on material fixed 42, 49, and 72 hours after treatment. Control material, fixed at each interval, was also examined. Only diploid cells were scored. All of the expected types of aberration were found in the irradiated material. A normal figure from control material is shown in Fig. 1*a*, and a figure containing a chromatid deletion is shown as Fig. 1*b*.

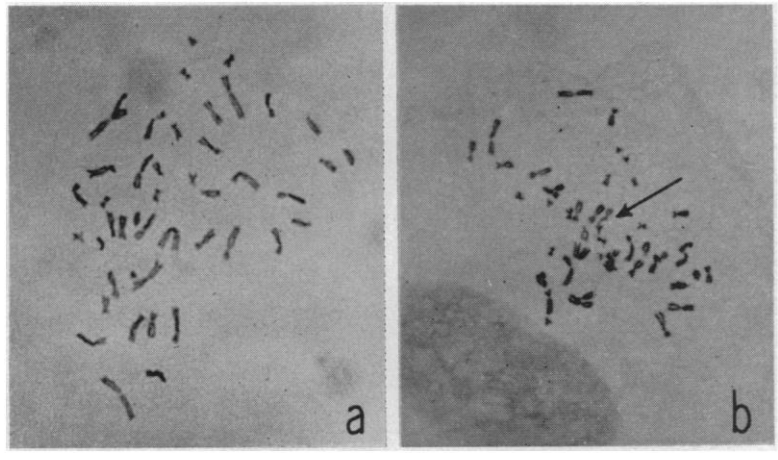

Fig. 1. Colchicine-treated metaphase figures from tissue cultured epithelioid diploid human kidney cells. (*a*) Normal figure from control material. (*b*) A figure from the 42-hour, 25-roentgen series, showing a chromatid deletion (arrow).

The results of the experiment are shown in Table 1. All of the samples smaller than 150 cells include all scorable cells in the sample. The 72-hour sample is included here in spite of its small size, because it shows a significant drop in the frequency of aberrations over the 42- and 49-hour samples, probably because the cells scored in the 72-hour sample were in early interphase at the time of irradiation. The differences between the irradiated samples and the controls for the 42- and the 49-hour samples are significant at the 1-percent level. The differences between the 42- and the 49-hour samples are not significant.

It is, of course, very obvious that before any definite conclusions can be drawn about the sensitivity of human tissues to radiation, the present preliminary work must be repeated on the same and other tissues. However, in the absence of other data and in view of the importance of the subject, it seems proper to point out that the present work indicates that human tissues may be much more sensitive to ionizing radiation than was previously suspected. If one combines the 42- and 49-hour data, the control rate is one break per 100 cells. The slope of the dosage versus breakage curve is about 0.3 break per 100 cells per roentgen. The doubling dose, for the types of aberrations scored and for this material, is thus about 3.3 roentgens. This is roughly one third of the maximum permissible dose recently recommended by the National Academy of Sciences' report on the Biological Hazards of Atomic Radiation. It should be pointed out that the National Academy's recommendation was based on estimates of gene mutations, which may not be as easily induced by x-rays as chromosome aberrations.

Work is in progress in this laboratory to determine induced gene mutation frequencies in normal diploid human cells *in vitro*. Although there is no proof that cells in tissue culture respond to radiation in the same manner as rapidly reproducing tissues in the body, there is no evidence that they do not. It is clear that if the rates of this and other types of radiation damage to human cells are found to be correspondingly high in further experiments, a sharp revision will have to be made in our estimates of "safe" doses of radiation, if, indeed, any dose can be called "safe" from a genetic point of view.

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4. I am indebted to the staff of the Brady Clinic and especially to its director, W. W. Scott, for this and other tissue specimens.
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2 October 1957

Pressure-Sensitive Telemetering Capsule for Study of Gastrointestinal Motility

Existing methods for the accurate measurement and recording of pressure changes within the human gastrointestinal tract require the passage of tubes through the mouth, nose, or anus, or through an artificial opening provided by gastrostomy, ileostomy, or colostomy. The principal objections to these methods are (i) that the distal small intestine and proximal colon are relatively inaccessible for study and (ii) that normal gastrointestinal motility may be altered by reflex changes induced either by the physical presence of the tube or by the discomfort experienced by the patient.

An instrument has been devised which will permit the recording of gastrointestinal motility under more physiologic conditions. This instrument is sensitive to intraluminal pressures and records these pressures without connecting wires or tubes. It consists of a rigid, plastic, cylindrical capsule 3.0 cm in length and 1.0 cm in diameter (Fig. 1). The capsule contains a transistor radio transmitter powered by a battery having a "life" of 15 hours. A screw-on cap at one end of the capsule permits replacement of the battery. The opposite end of the capsule is a flexible rubber membrane which covers a pressure transducer. Pressure applied to the transducer modulates the frequency of the oscillations generated by the transmitter. These signals are accepted by the antenna of a frequency-modulation receiver. The receiver demodulates the signals, and the pressure variations are displayed on an oscilloscope and recorded photographically. The capsule detects pressures ranging from 0 to approximately 50 cm of water and responds to frequencies between 0 and 100 cy/sec.

This pressure-sensitive device has been used to record pressures within the gastrointestinal tract in normal human subjects. Prior to ingestion of the capsule, the entire detecting and recording apparatus is calibrated in an external system. The capsule is placed in a bottle, which is rendered air-tight by a two-hole stopper. One opening admits a water manometer, and the other permits injection of increments of air. The pressures developed within the bottle are recorded in the usual fashion by means of the frequency-modulation receiver and the antenna which is placed near the bottle. A given excursion of the photographic record corresponds to the pressure change indicated by the water manometer. Such calibration permits accurate derivation of relative intraluminal pressures from the final record.

The recording of gastrointestinal pressures in man is accomplished with the subject in any comfortable position and with the antenna secured loosely to the abdomen. Respirations are recorded simultaneously by a pneumograph attached to a strain-gage manometer. Both the intraluminal pressures and the respiratory excursions are recorded by a multichannel photographic recorder and displayed on an oscilloscope. The capsule may be swallowed without difficulty, and it passes through the gastrointestinal

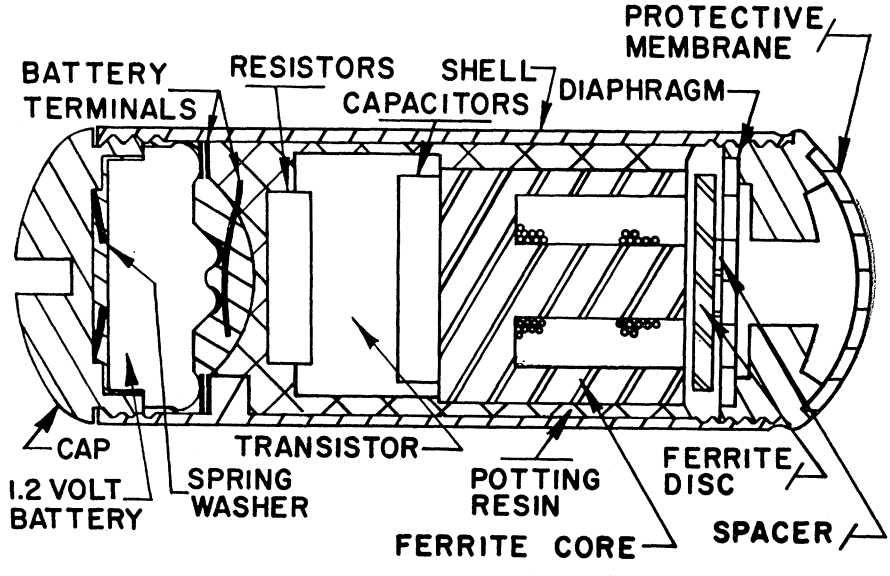

Fig. 1. Cross-section of the pressure-sensitive radio transmitter.