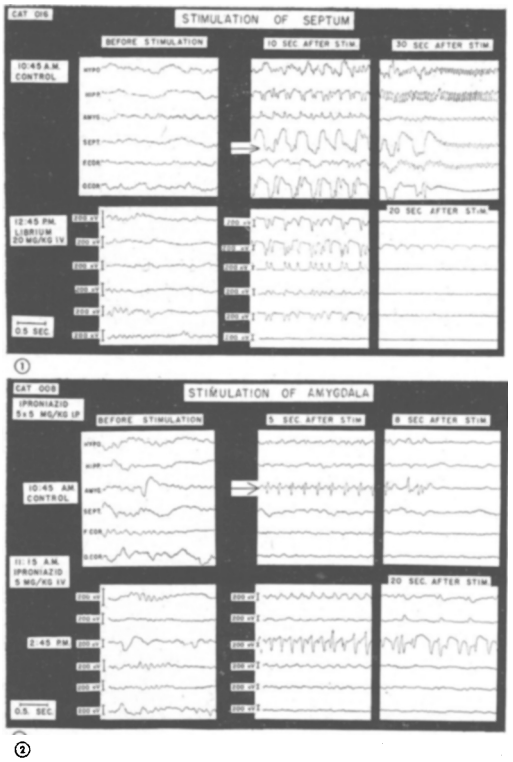

FIG. 1. Chlordiazepoxide, 20 mg/kg i.v., decreases duration of discharge in septum. Upper row is before, lower row 30 min. after, drug inj. First panel in each row is before, second and third panels after, electrical stimulation of area indicated by arrow. Note reduced amplification in second and third panels. EEG leads are hypothalamus, hippocampus, amygdala, septum, frontal and occipital cortex. Paper speed 6 cm/sec.

FIG. 2. Iproniazid, 5 mg/kg i.v. in cat pretreated for 5 days with 5 mg/kg i.p., increases duration of discharge in amygdala. Arrangement as in Fig. 1.

increases aggressiveness, reduced activity of this area might have a psychodepressant effect.

We found that chlordiazepoxide at 10 mg/kg i.v. had depressant effects on septum, amygdala and hippocampus. In other tests, this compound was found to be more potent than meprobamate in depressing the irritability of rats with septal lesions, and in reducing the aggressiveness of vicious monkeys (4). In the clinic, this agent "reduces anxiety and agitation but does not cloud consciousness or impair intellectual function"(5). Perhaps these psychodepressant effects are correlated with depression of the amygdala; much more experimentation is needed before any such relationship can be established.

Summary. Four psychotropic drugs had the following statistically significant effects on the limbic system of the cat: iproniazid increased duration of after-discharge of amygdala; imipramine and meprobamate decreased duration of discharge of septum; chlordiazepoxide decreased duration of discharge of septum and hippocampus, and decreased amplitude of discharge of amygdala.

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Effects of Selective Ultraviolet Irradiation of Cytoplasm of Living Cells.* (26029)

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The authors have reported some of the manifestations of ultraviolet irradiation damage in living cells(1,2). The effects reported concerned the nature of the response of the whole cell to ultraviolet irradiation, rather

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than effects of ultraviolet irradiation of selected portions of living cells. The purpose of this report will be to extend previous observations by recording additional types of ultraviolet induced irradiation damage in living cells, and to record some observations on response of the cytoplasm alone to ultraviolet irradiation.

Methods. We have previously reported the technic of ultraviolet flying spot television microscopy and time-lapse cinematography (3,4, 5). Recently the authors have recorded improvements in this technic which permit use of double flying spot illumination (6). In the experiments reported here the principle of double flying spot illumination was employed to illuminate the nucleus of the cell with light of a peak wave length of 4500 Å and to illuminate the cytoplasm of the same cell with ultraviolet light with a peak wave length of 2600 Å. Control cells in the same microscopic field were illuminated solely with ultraviolet light. The ultraviolet emitting scanner tube and the visible light emitting scanner tube—which in this case acted as the sources of illumination — were permitted to operate continuously at a scan rate of 4 frames per second. Every 7 seconds the monitor tube was selectively blanked so that a new 16 mm film frame could be pulled into position. Each 16 mm film frame stored the image information from 28 scanned frames of the monitor tube. By storing the monitor tube information from multiple scan frames on one 16 mm film frame the signal to noise ratio was greatly increased. During the experiments the equipment was periodically altered so that a complete ultraviolet absorption image of all cells was obtained. This ultraviolet absorption image was obtained and recorded on the film for several minutes at selected intervals and again for 30 minutes at end of experiment. Three types of cells were used in this experiment; HeLa cells, Chang Liver cells, and mouse fibroblasts. All of the cells gave identical results. All of the cells were carried in stock cultures in Eagle's media enriched with horse serum and for purposes of the experiment they were transferred to Rose chambers with quartz coverslips. During the course of the experiment only, the chambers were supplied with Eagle's media without horse serum. Horse serum was removed from the media during experiment because its absorption of ultraviolet light at these wave lengths obscured the nature of the absorption image of the cells. In all, these results represent data taken from 5,000 feet of 16 mm black and white film. A total of 1,000 cells was studied. Fifty of these were subjected exclusively to cytoplasmic ultraviolet irradiation while the remaining 950 cells acted as controls and were subjected to total cell ultraviolet irradiation.

Results. Ultraviolet irradiation applied to the whole living cell will result in a variety of damage patterns depending on dose and rate of dosage. The most acute pattern of this response is a sudden collapse of the cell from its normal spread-out attachment to the coverslip into a round ultraviolet opaque ball without attachment to the coverslip. This sudden collapse occurs at the end of 1 hour of scanning at a scanner tube beam current of 50 microamps and is entirely unheralded by a change in absorption of the cell or by a change in its motion. With less intense doses of irradiation given by a scanner tube beam current of 20 microamps several types of damage appear. These may be divided into cytoplasmic components and a nuclear component. The most subtle form of cytoplasmic damage consists of a gelling of the cytoplasm. This has 2 effects on cytoplasmic motion. One effect is to increasingly restrict, to the point of cessation, the motion of lipoprotein droplets of the cytoplasm. The second effect is completely to inhibit pinocytosis so that all movement of the cytoplasmic membrane is lost. These effects take place on the average after 2 hours of scanning. During the next several hours other cytoplasmic effects follow, consisting of several varieties of bubble formation. One type of bubble is a large nonabsorbing bubble forming on/or from the cytoplasmic membrane. These bubbles are apparently formed by syneresis. A second type of bubble differs from the first in that the bubbles in this instance are filled with cytoplasm and contents of these bubbles show ultraviolet absorption comparable to the remaining cytoplasm. Still a third type of bub-

ble which may follow development of the first two is the intracytoplasmic bubble. This type of bubble is usually multiple and is always transparent to ultraviolet light. All 3 types of bubbles have been observed to rupture and release their contents into the environs. In the end the cytoplasm loses virtually all of its absorption and it decreases markedly in area. During the time interval required for production of these cytoplasmic manifestations of damage the nucleus shows marked evidence of damage. In the undamaged cell the nuclear membrane is not visible as an ultraviolet absorbing structure; the nuclear sap shows slight ultraviolet absorption on the order of 10% of absorption of the cytoplasm adjacent to the nucleus, while the nucleolus shows intense absorption. As cellular damage due to ultraviolet irradiation progresses, a condensation of ultraviolet absorbing material takes place at the nuclear membrane. The nucleus progressively diminishes in size to approximately one-half its original state and the nuclear membrane appears to become more and more prominent. As the nucleus decreases in size, the increasingly prominent nuclear membrane becomes wrinkled and infolded. Together with these changes the nucleus displays a general increase in absorption, roughly paralleling the progressive decrease in cytoplasmic absorption. There is, however, no proof that the two are related other than in time. The increase in nuclear absorption may be as much as 4-fold. During these processes of cellular damage the nucleoli do not change size, shape or absorption.

When the experiment is arranged so that cytoplasm alone is irradiated by ultraviolet, the following results were noted. The cytoplasm of the cell with the protected nucleus shows the same responses to ultraviolet irradiation as does the cytoplasm of control cells in the field. These are the responses detailed in the preceding section, and they occur at the same time in cytoplasm of control cells in the field of irradiation as in the cell with the nucleus illuminated with visible light. Nuclear responses in protected cell and in control irradiated cells are quite different. Nuclei of the totally irradiated control cells show the typical nuclear responses to irradiation detailed in the preceding section. Nuclei of the protected cells do not show any of these responses. Their only change during experiments is a change in shape which accompanies the changing shape of the cell as the cytoplasm bubbles and finally retracts to give the cell a rounded shape. Nuclear absorption of ultraviolet light at beginning, middle and end of the experiment remains the same as an unirradiated cell. The nuclear membrane never becomes an ultraviolet absorbing structure even in the face of the most severe cytoplasmic damage. Nucleoli in the protected nuclei remain unchanged in number, size, shape and ultraviolet absorption.

Discussion. Previous experiments in the literature dealing with the role of the nucleus in ultraviolet irradiation damage have been carried out differently from those reported here. These studies have involved single relatively large doses of irradiation to intact amoeba, or to enucleated amoeba, or to separated amoeba nuclei. The latter may be suitably recombined with cytoplasm to study the effects of transplanted irradiated or unirradiated nuclei, grafted into irradiated or unirradiated cytoplasm. Under these circumstances the unirradiated nucleus grafted into irradiated cytoplasm produces an increase in longevity as compared with irradiation of the intact organism(7,8,9,10,11). The experiment reported here differs so markedly from the above experiment that no comparison is possible. One of the major areas of variance is that in the experiment reported here the cytoplasm was continuously irradiated until damage appeared. A second difficulty rests in the fact that no existing technic may be applied to determine dose of irradiation given to the specimen by the flying spot microscope. Hence it is not possible to compare irradiation dosimetry.

It would appear from these experiments that continuous ultraviolet irradiation of the cytoplasm of the intact cell produces the same cytoplasmic effects as does continuous irradiation of the entire cell. In these experiments nuclear evidence of ultraviolet induced irradiation damage did not occur.

Conclusions. (1) Nuclear protection from

ultraviolet irradiation during continuous ultraviolet irradiation of cytoplasm confers no degree of protection on cytoplasm or on longevity of the cell. (2) Nuclear protection from ultraviolet irradiation during continuous ultraviolet irradiation of cytoplasm prevents nuclear manifestations of ultraviolet induced nuclear damage.

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Constancy of Gastric Acidity with Variable Secretory Rates Induced by Insulin or Glucagon, with Histamine.* (26030)

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There is now considerable evidence to controvert the old concept concerning gastric acid secretion according to which concentration of acid in the primary secretion, as it arises from the parietal cell, varies with volume rate of flow(1). In the experience of our group, it has been found that the acidity of pouch juice may remain constant at high levels (*e.g.*, in the neighborhood of 150 meq/l) for significantly long periods of time and under a variety of experimental conditions. This was first observed in dogs with Pavlov pouches, following a single subcutaneous injection of histamine of very high dosage; in these experiments, acidity maintained a plateau level for a long time, during which rate of secretion rose and then fell to a level of about 10% of its maximum(2). Similar evidence has been adduced from secretion induced by low dosages of histamine and by ingestion of food, when the juice was collected in such a way as to minimize the concomitant secretion of mucus(2); also during the diurnal hyper-secretory activity manifested by a bitch during lactation(3). Again, in experiments with Heidenhain pouch dogs in which both acidity and volume-rate were maintained at a fair degree of constancy, by repeated injections of histamine at short intervals (0.025 mg base every 10 minutes) for many hours, the superimposition of a single subcutaneous injection of atropine (0.4 mg/kg) about the middle of a 4-6 hour run, in order to inhibit the secretory response to the continuing administration of histamine, resulted in a drastic reduction in volume-rate without any marked change in acidity(4).

At no time, however, has it been reported that such essential lack of correlation between acidity and volume-rate obtains with purely vagal or vagomimetic stimuli, or even histamine at low dosage under ordinary collection conditions, but this may be ascribed to the fact that such stimuli evoke a gastric juice which is relatively high in organic matter—enzymes and mucus—and therefore relatively low in acidity. As a result of variations in these organic constituents, constancy of acidity is practically never attained, and time

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