low thromboplastic activity in intima of vena cava. Though we observed fibrinolytic activity in most of the samples of pulmonary arterial intima (9 of 14) intimal activity in most samples of pulmonary vein was considerably higher and none was zero. The discrepancy between this finding and the observation by Todd(5) could possibly be due to the fact that he investigated small arteries and veins present in sections of lung tissue. We could confirm with our extraction method his histological finding of fibrinolytic activity in the intima of large systemic veins (vena cava inferior), which in some cases was even higher than in the corresponding sample of adventitia.

Summary. Thromboplastic and fibrinolytic activity was estimated in layers of large human vessels. Arterial intima has higher thromboplastic and lower plasminogen activator concentrations (usually zero) than the intima of large systemic veins (vena cava inf.). In all vessels adventitia has high fibrinolytic activity.

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Effects of Selective Ultraviolet Irradiation of the Nuclei of Living Cells.* (26942)

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The authors have reported some of the manifestations of ultraviolet irradiation damage in living cells(1,2,3). The effects reported concerned the nature of the response of the whole cell to ultraviolet irradiation, and the effects of selective ultraviolet irradiation of the cytoplasm of living cells. The purpose of this report is to extend the previous observations by recording an additional type of ultraviolet irradiation of a selected area of the cell. This report deals with the nature of the damage produced in intact living cells by selective ultraviolet irradiation of the nuclei of these cells.

Methods. We have previously reported the technic of ultraviolet flying spot television microscopy and time-lapse cinematography (4,5,6). Recently the authors have recorded improvements in this technic which permit the use of double flying spot microscopy illumination(7). 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 2600 Å and to illuminate the cytoplasm of the same cell with light of a peak wave length of 4500 Å. Control cells in the same field were illuminated solely with light of 4500 Å wave length. Although it was impossible to irradiate the nuclei of the cells without including a small amount of cytoplasm in the irradiated area, it is felt by the authors that this amount of irradiated cytoplasm was so small in comparison to that which was protected from the irradiation that its effects were negligible.

No presently available technic could be applied to determine the dose of irradiation given to the nucleus of the cell.

The ultraviolet emitting scanner tube and the visible light emitting scanner tube—which in this case acted as the source of il-

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lumination—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. At the end of the experiments the equipment was 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.

The cell type used in this experiment was Chang Liver. 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 the 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 61 cells was studied.

Results. Continuous ultraviolet irradiation of the nuclei of living cells for 14 hours or more with a beam current of 25 microamps applied to the ultraviolet emitting cathode ray scanner tube produces a variety of types of damages to the cell. These effects may be divided into nuclear and cytoplasmic types.

The morphologic aspects of the nuclear effects of nuclear ultraviolet irradiation are not pronounced. The nuclear membrane often becomes more prominent in the ultraviolet field, due probably to the condensation of ultraviolet absorbing material or particulate material around it. This effect is often attended by a slight increase in ultraviolet absorption by the nucleus. These changes are observed at the end of approximately 8 hours of irradiation.

The nucleoli do not change in appearance or in amount of ultraviolet absorption during the experiment.

The cytoplasmic changes which accompany nuclear irradiation consist first of a gelling of the cytoplasm which occurs after an average of 2 hours' irradiation. This gelling is evidenced by a stasis of lipoprotein particles in the cytoplasm coupled with a cessation of pinocytosis.

After about 4 hours' irradiation, a gradual decrease in cytoplasmic area occurs, extending over several hours. The most striking evidence of the decrease in area is a thinning and withdrawal of cytoplasmic streamers as the cell attains a more nearly spherical shape.

Bubbling of the cytoplasm occurs in less than 10% of the cells during nuclear irradiation. Three different types of bubbling are seen. Our type is a large nonabsorbing bubble forming by syneresis on or from the cytoplasmic membrane. A second type differs from the first in that the bubbles are filled with cytoplasm and the contents of these bubbles exhibit ultraviolet absorption comparable to the remaining cytoplasm. Still a third type is the intracytoplasmic bubble. This type of bubble is usually multiple and is always transparent to ultraviolet light. All 3 bubbles have been observed to rupture and release their contents into the environs. One or more of these types of bubbles are observed in different cells; however, all 3 types are never seen in the same cell. When bubbling occurs, it is usually after prolonged periods of nuclear irradiation, after 14 hours or more.

Ultraviolet absorption images of the entire cell after several hours of nuclear irradiation reveal no decrease in ultraviolet absorption of the cytoplasm as compared to unirradiated cells in the field.

Two additional effects of nuclear irradiation may be noted: 1) In 10% of the experiments, free cells which happen to be in the vicinity of the cell which was undergoing nuclear irradiation tend to migrate toward, and coalesce around, the latter cell. 2) In none of the instances observed did a cell undergoing nuclear irradiation go into mitosis.

Discussion. As previously reported(2) ultraviolet irradiation applied to the whole cell

by these technics may take several patterns, briefly summarized as follows. The most acute pattern is a sudden collapse of the cell into a round ultraviolet opaque ball. With less intense doses, the cytoplasm first begins to gel, then may display a variety of bubbles and lose its characteristic absorption. Coupled with these latter phenomena the nuclear membrane increases in prominence as an ultraviolet absorbing structure and begins to wrinkle as the nucleus collapses. During this period the nucleus itself begins to decrease in size and increase in absorption and may finally decrease in diameter by as much as 50% and incerase in absorption by as much as 4-fold. During these processes of cellular damage, the nucleoli do not change their size, shape or absorption.

As previously reported(3), nuclear protection from ultraviolet irradiation during constant ultraviolet irradiation of the cytoplasm prevents the nuclear manifestations of ultraviolet induced cellular damage, while conferring no degree or type of protection on the cytoplasm or on the longevity of the cell.

The cytoplasmic effects of nuclear irradiation resemble, in a lesser degree, those effects elicited by irradiation of the entire cell. The gelling of the cytoplasm is quite similar in both cases; however, bubbling of the cytoplasm is much more consistent in both frequency and type during irradiation of the entire cell than during irradiation of the nucleus alone. The sudden collapse of the cell which occurs when the entire cell is irradiated does not occur during nuclear irradiation; rather the cytoplasm withdraws gradually, and never collapses completely.

Nuclear effects of nuclear irradiation resemble those changes which occur during irradiation of the entire cell, but to a lesser degree. The most striking difference is that during nuclear irradiation marked shrinkage and collapse of the nuclei do not occur. The increased prominence of the nuclear membrane is less during nuclear irradiation and the increase in ultraviolet absorption by the nucleus is not so marked.

During nuclear irradiation, the nucleoli are not observed to change in size, shape, number, or ultraviolet absorption. In a previous experiment in which the nucleoli alone were irradiated, the nucleoli were observed to lose 90% of their characteristic absorption after 6 to 8 hours of ultraviolet irradiation(8). This loss of absorption is presumably due to an alteration of the protein and nucleic acid composition of the nucleolus; this alteration apparently does not occur when the entire nucleus is irradiated.

Previous experiments from the literature involving nuclear irradiation have differed markedly from the above experiments. The most closely analogous experiment dealt with the effects of ultraviolet irradiation of the nuclei of living amoeba(9). In this experiment, it was found that when nuclei of irradiated cells are transferred to unirradiated cells, not only is the cytoplasm of the unirradiated cell unable to reverse the damage to the irradiated nucleus, but the unirradiated cytoplasm itself is damaged by contact with the irradiated nucleus.

From the results of a similar experiment on isolated thymus and liver nuclei(10), it was concluded that cytoplasmic recovery from x- and uv-irradiation can occur with intact nuclei, with the reverse being much less obvious. The cytoplasm was not seen to be more radioresistant than the nucleus, however.

Other experiments(11,12) have dealt with the effects of uv-irradiation on cells in various stages of mitosis. It is reported that chromosomal aberrations have been induced as well as a cessation of normal mitotic progression.

Other experiments(13,14) deal with the effects of x-rays and alpha particles on DNA synthesis and mitosis in ova and other cells. It is reported(14) that a temporary decrease in DNA synthesis occurs in such cells, followed by a resumption of DNA synthesis in which the cells do not divide and giant cells are produced. Apparently, these effects are due to the irradiation of the nucleoli of the cells rather than the nuclear sap itself(14).

The reported experiments differ so greatly from the above experiment that no comparison of results is possible.

Conclusions. (1) Continuous ultraviolet irradiation of the nucleus of a living cell

produces nuclear damage. Although this damage resembles nuclear damage which occurs when the entire cell is irradiated, it is less severe due to the protection of the cytoplasm from the effects of irradiation. (2) Continuous ultraviolet irradiation of the nucleus of a living cell produces cytoplasmic damage resembling that which occurs when the entire cell is irradiated, but is less pronounced.

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Septal and Anterior Hypothalamic Lesions: Some Effects on Sleep and Body Metabolism in Cats.* (26943)

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It is currently hypothesized that the posterior hypothalamus and ascending reticular formation are concerned with the state of wakefulness. In accordance with this, it has been postulated that somnolence is due to periods of depressed activity within these regions. Some authors, although supporting the relationship of these areas to wakefulness, have preferred to regard these periods of depressed function as due to influences from specific mechanisms which may be subject to extrinsic rather than intrinsic factors.

Ranson and Ingram(1), and again Ingram *et al.*(2) reported that lesions placed in a number of areas of the cat's hypothalamus caused somnolence, but no sleeplessness was noted. Kabat *et al.*(3), stimulated the preoptic region of cats without producing sleep. Ranson and Magoun(4) partly destroyed this latter region (in cats) and observed no change in sleep-wakefulness cycles. Insomnia was not mentioned by Ranson(5) after lesions in the hypothalamus of monkeys.

Nauta(6) described a condition of sleeplessness produced by elimination of the suprachiasmatic and preoptic areas in rats. Postoperatively these animals behaved more or less normally, but would not sleep and after a few days lapsed into a state of exhaustion and died. Nauta concluded that there might be an area in the preoptic region which served as a "sleep center", as opposed to a "waking center", referring to a region in the posterior hypothalamus around the mammillary nuclei. This latter area, when removed, would produce somnolence, and Nauta believed that the "sleep center" functionally inhibited the "waking center" to produce a state of sleep. Heath(7) placed lesions somewhat rostral to those of Nauta, but including some areas in common, and no sleeplessness was reported.

Considering Nauta's findings from the standpoint of metabolic and hypothalamic re-

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