Dr. Willys Silvers, University of Pennsylvania, Philadelphia.
7. Complete medium consisted of minimum essential medium (Eagle) with Earle's salts supplemented with 10 percent heat-inactivated fetal calf serum, 0.5 percent lactalbumin hydrolysate, 2 m*M* L-glutamine, and (per milliliter) 100 units of penicillin, 100 μg of streptomycin, and 0.25 μg of fungizone. All tissue culture reagents were obtained from Grand Island Biological Company, Grand Island, New York.
8. Falcon Plastics, division of B-D Laboratories, Inc., Los Angeles, California.
9. G. D. Hsiung, *Diagnostic Virology* (Yale Univ. Press, New Haven, Connecticut, 1964), p. 7.
10. *Mycobacterium tuberculosis*; Bayol F from Humble Refining Company, Bayonne, New Jersey.
11. Egg albumin (5× crystalline, Nutritional Biochemicals Corp., Cleveland, Ohio), 2 mg per milliliter of saline emulsified with equal amounts of complete Freund's adjuvant [15 percent Arlacel A (Atlas Powder Co., Wilmington, Delaware), 85 percent Bayol F, and 6 mg of tubercle bacilli per milliliter].
12. Parke-Davis, Detroit, Michigan.
13. Trypsin (0.25 percent) in a balanced salt solution containing 0.02 percent ethylenediaminetetraacetic acid.
14. Coulter counter, model A (Coulter Electronics Co., Hialeah, Florida).
15. B. H. Waksman, in *Mechanism of Cell and Tissue Damage Produced by Immune Reactions* (Second International Symposium on Immunopathology, 1961) (Grune & Stratton, New York, 1962), p. 146.
16. We thank Dr. Frank Ruddle for assistance in establishing the tissue culture lines. From a thesis submitted to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Presented in part at the meetings of the American Society of Microbiology, 1 May 1967, New York. Supported by NIH grants AI 06112 and AI 06455. N.H.R. was supported by PHS training grant 5 T1 AI 291.

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Inhibition of Lipolytic Action of Growth Hormone and Glucocorticoid by Ultraviolet and X-Radiation

Abstract. *Addition of catecholamines or growth hormone plus glucocorticoid activates lipolysis in isolated white-fat cells of the rat. If the fat cells were irradiated just before the addition of growth hormone and glucocorticoid, their lipolytic action was abolished. However, irradiation did not affect the activation of lipolysis by catecholamines.*

The addition of growth hormone (0.01 to 0.1 μg/ml) in the presence of glucocorticoid to free white-fat cells increases lipolysis after a lag period of 1 to 2 hours (*1*). The lipolytic action of growth hormone is abolished by inhibitors of protein synthesis, such as puromycin and cycloheximide, and by actinomycin which blocks DNA-dependent RNA biosynthesis (*1*). Catecholamines also accelerate fat-cell lipolysis, but their effect is rapid in onset and is not affected by any of these antibiotics (*1*). It is thought that catecholamines increase the concentration of cyclic 3′,5′-AMP (adenosine monophosphate) which activates the lipase involved in hydrolysis of triglycerides to glycerol and free fatty acids (*2*). Regulation of fat-cell lipolysis appears to be the major site for hormonal control of lipid mobilization and is thus of importance in the metabolic adjustments to caloric deprivation of animals.

We now report that ultraviolet light and x-radiation have the same effect as actinomycin and cycloheximide on the lipolytic action of hormones. The results support the hypothesis that RNA and protein synthesis are involved in the lipolytic action of growth hormone but not in that of catecholamines.

Free fat cells were isolated from the parametrial adipose tissue of starved female rats. The adipose tissue from three to six rats was incubated for about 45 minutes in Krebs-Ringer phosphate buffer containing 0.5 mg of crude bacterial collagenase per milliliter and 1 percent albumin. Samples of the dissociated adipose tissue were taken for exposure to ultraviolet light or x-radiation. Immediately after irradiation, the suspension of cells and tissue debris was centrifuged, and the medium with contaminating cells was removed by aspiration. The free fat cells were washed twice with albumin buffer and then placed in plastic flasks containing 3 percent bovine fraction-V albumin (Pentex No. 55) and 2.4 m*M* glucose in phosphate buffer; this cell suspension was shaken for 4 hours at 37°C in an incubator. The gas phase was air. Initial control values were obtained with fat cells incubated for 5 minutes, and all incubations were performed in duplicate.

Table 1. Effect of ultraviolet light and x-radiation on incorporation of leucine into protein and of uridine into RNA by fat cells, expressed as percent of inhibition. The values are shown as the mean ± S.E. of the percentage of inhibition due to ultraviolet light or x-radiation and represent seven paired replications. The basal value for incorporation of L-leucine-4,5-H^3 into protein was 1.26 percent of added label per gram of fat cells; for uridine-5-H^3, it was 1.39 percent. An average of 15 to 25 mg of cells were incubated for 4 hours in a volume of 2 ml.

Treatment	Inhibition (%)	
	Leucine into protein	Uridine into RNA
Ultraviolet (1 min)	50 ± 20	40 ± 14
Ultraviolet (2 min)	52 ± 20	70 ± 7
5000 rad	10 ± 16	80 ± 9
10,000 rad	27 ± 15	69 ± 10

Glycerol was analyzed by a fluorometric micromodification of the procedure of Vaughan (*3*) on samples of the medium and free fatty acids by a modification of the titrimetric procedure of Dole and Meinertz (*4*). The amount of fat cells present in each flask was based on total fatty acid content (*1*). Incorporation of L-leucine 4,5-H^3 (5 c/mmole) into protein and uridine-5-H^3 (5 c/mmole) into RNA was determined by a modification of the described procedures (*1*). However, RNA synthesis was based on uridine incorporation into material precipitable by trichloroacetic acid.

Fat cells were irradiated with a Picker x-ray generator operating at 280 kv and 20 ma. The beam was filtered with 0.4 mm of Sn, 0.25 mm of Cu, and 0.5 mm of Al. The dose rate was 1000 rad per minute, and the cells were placed in a plastic cup on a lead plate with air as the gas phase.

Ultraviolet irradiation was performed with a Hanovia No. 24,550 lamp which primarily emits light at 2537 Å. The cells were placed 33 cm below the light and exposed for 1 or 2 minutes. At this distance, 1-minute exposure inactivated 99.9 percent of a suspension of T3 bacteriophage, as based on plaque-forming ability.

Previously, a significant amount of basal lipolysis occurred, and the addition of bovine growth hormone (NIH-GH-B3 or B6) in the presence of 9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadien-3,20-dione (dexamethasone, a potent synthetic glucocorticoid) accelerated lipolysis in fat cells from starved rats without added catecholamine (*1*). In these studies (Figs. 1 and 2), there was neither basal lipolysis by fat cells obtained from fasted rats nor lipolytic response to growth hormone and dexamethasone in the absence of added catecholamine. These differences are due to the finding that currently available commercial preparations of bovine fraction-V albumin contain an inhibitor of fat cell lipolysis (*5*). The effects of the inhibitor can be overcome by adding catecholamine; the increase in lipolysis due to growth hormone and dexamethasone was the same in the presence of either 0.5 or 0.05 μg of epinephrine per milliliter (*5*).

The results in Fig. 1 indicate that

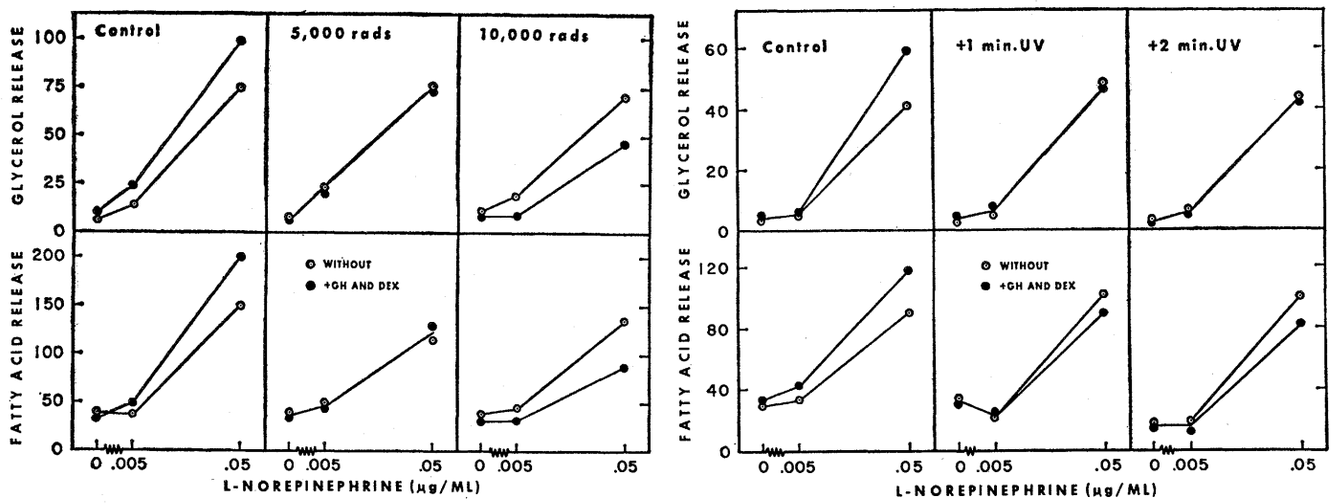

Fig. 1 (left). Effect of x-radiation on hormonal activation of lipolysis in free fat cells. (Left) 9.6 mg of cells per tube; (middle) 8.5 mg of cells per tube; (right) 8.0 mg of cells per tube. The cells were incubated for 4 hours in a volume of 2 ml. *GH*, growth hormone at a concentration of 0.5 µg/ml; *Dex*, dexamethasone at a concentration of 0.016 µg/ml. Fatty acid and glycerol release are expressed in micromoles per gram of cells. Values are the means of eight paired replications. In the presence of 0.05 µg of L-norepinephrine per milliliter, lipolysis was significantly stimulated by growth hormone and dexamethasone in the unirradiated controls and depressed by these hormones in the cells exposed to 10,000 rad ($P < .05$ by paired comparisons). There was no significant effect of either dose of radiation on the response of cells to L-norepinephrine. Fig. 2 (right). Effect of ultraviolet light on fat-cell lipolysis. (Left) 8 mg of cells per tube; (middle) 8 mg of cells per tube; (right) 8.5 mg of cells per tube. *GH*, growth hormone at a concentration of 0.5 µg/ml; *Dex*, dexamethasone at a concentration of 0.016 µg/ml. Values are the means of seven paired replications. In the presence of 0.05 µg of L-norepinephrine per milliliter, lipolysis was significantly stimulated by growth hormone and dexamethasone in the unirradiated controls ($P < 0.5$ by paired comparisons).

5000 rad of x-radiation completely blocked the increase in both glycerol and fatty acid release due to growth hormone and glucocorticoid in the presence of 0.05 µg of norepinephrine per milliliter. In cells exposed to 10,000 rad, growth hormone and glucocorticoid actually inhibited lipolysis. Similar insulin-like effects of growth hormone on glucose metabolism, observed in vitro with adipose tissue from hypophysectomized rats, are not blocked by actinomycin (*6*).

There was no significant effect of either dose of x-radiation on the lipolytic action of norepinephrine or on basal lipolysis. Microscopic examination of irradiated fat cells stained with methylene blue indicated that the dosages used did not result in appreciable cell breakage. Fat cells can be readily lysed with surface-active agents such as Triton X-100, but these cells do not show a lipolytic response to catecholamines (*5*).

The effect of ultraviolet light on fat-cell lipolysis was almost identical to that of x-radiation (Fig. 2). Neither the lipolytic response to norepinephrine nor basal lipolysis was affected by exposure to ultraviolet light for 1 or 2 minutes. However, the increment in lipolysis due to growth hormone and glucocorticoid was completely abolished.

In view of the fact that ultraviolet light and x-radiation blocked the lipolytic action of growth hormone, their effect on RNA and protein synthesis was examined. The x-radiation (5000 or 10,000 rad) and exposure for 2 minutes to ultraviolet light markedly reduced RNA synthesis, while exposure to ultraviolet light for 1 minute had a lesser effect (Table 1). There was no significant effect of x-radiation on leucine incorporation into fat-cell protein, while ultraviolet light reduced leucine incorporation into protein by 50 percent (Table 1). The effects of x-radiation on protein and RNA synthesis in fat cells are similar to those reported for actinomycin (*1*). The preferential inhibition of RNA synthesis by x-radiation confirms previous studies of RNA and protein formation in intestine and bone marrow of rabbits (*7*).

In contrast to these results, actinomycin and x-radiation have different effects on the elevation of enzyme activities in vivo by substrates and hormones (*8*). The elevation of hepatic tryptophan pyrrolase and tyrosine transaminase by administration of glucocorticoid in vivo to rats was unaffected by low doses of x-radiation and was blocked by actinomycin. The activation of these two enzymes by substrates was blocked by whole-body x-radiation but not by actinomycin (*8*). The amounts of x-radiation (1600 roentgens or less) used in the above studies have little effect on total RNA synthesis in normal liver (*9*). However, substrate-type elevation of hepatic enzyme activities does not appear to involve DNA-dependent RNA synthesis (*8*).

Ultraviolet light and x-radiation prevent the formation of induced enzymes in microorganisms (*10*). The evidence suggests that DNA-dependent RNA synthesis is more sensitive to x-radiation than to ultraviolet light, while the opposite may hold for protein synthesis (*11*). This is in accord with my findings that protein synthesis in fat cells was preferentially inhibited by ultraviolet light (Table 1). The reverse was true for RNA synthesis, particularly for the lower dose of ultraviolet radiation (Table 1). Possibly, ultraviolet radiation and x-radiation affect aspects of fat-cell metabolism other than protein and RNA synthesis. However, the similarity in the action of x-radiation to that of actinomycin, and of ultraviolet light to that of puromycin and cycloheximide, supports the hypothesis that RNA and protein synthesis is involved in the lipolytic action of growth hormone and glucocorticoid in white-fat cells.

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Herpes-Type Virus and Chromosome Marker in Normal Leukocytes after Growth with Irradiated Burkitt Cells

Abstract. *Cultured cells derived from male patients with Burkitt's lymphoma and harboring herpes-type virus particles were lethally irradiated. These irradiated cells induced normal peripheral leukocytes of female infants to grow within 2 to 4 weeks after mixed cultivation. Cells of a line free of this agent failed to stimulate growth. If either type of cell was cultured separately, it did not survive under the experimental conditions. Herpes-type viral antigen and C-group chromosomal marker previously described in cultured Burkitt cells were found in all of the female cell cultures that were obtained.*

A small number of cells in most continuous cultures derived from Burkitt lymphomas harbor herpes-like virus particles (*1–3*), as judged from electron-microscopic examination. Apparently, the same type of virus has also been found in some cell lines established from peripheral leukocytes of leukemic patients (*4*) or even of healthy donors (*5*). The virus-producing cells in these cultures can readily be detected by indirect immunofluorescence tests not only with serums from patients with Burkitt lymphoma, but with many other human serums, regardless of source (*6, 7*). By various procedures, it was shown that the fluorescent cells contain virus particles (*8*), that serums which yield positive immunofluorescence have antibodies to the viral capsid (*9*), and that the agent represents a hitherto-unknown member of the herpes group of viruses (*6, 10*). Whether this widely disseminated virus is merely a passenger in these cultures or whether it plays a role in the etiology of lymphomas, leukemias, or any other human illnesses is unknown. Attempts to transmit this agent to other host systems have not been successful, possibly because most of the virus particles are defective (*1, 3, 11*). Nor has it been possible to induce tumors in animals or to transform normal cells in vitro by addition of intact or disintegrated cell suspensions or by virus particles extracted from such suspensions. The presence of a chromosomal marker in cultured Burkitt cells has been described (*12, 13*); this marker consists of a subterminal constriction or achromatic gap on the long arm of one or both No. 10 chromosomes. There is some evidence that the same marker may also be found in some of the cell lines derived from leukemic patients (*13, 14*). The cause for this abnormality has not been established, but it could be due to the herpes-type virus associated with Burkitt and other cell lines, as indicated by our current finding that the herpes-type virus can be transmitted to normal peripheral leukocytes and that it may stimulate their growth.

Since infections by certain viruses of the herpes group are spread predominantly by close cell-to-cell contacts rather than by dissemination of virus via the fluid medium, it was desirable to explore the effects of nonreplicating Burkitt cells on other human cells on mixed cultivation with the use of the chromosomal sex of the two types of cells as marker for identification of outgrowing cell populations. The general techniques were as follows. Lethally x-irradiated (3000 to 6000 roentgens) cells from two Burkitt lines of cells derived from males served as "donors"; one harbored herpes-type virus particles in 3 to 5 percent of the cells (Jijoye), the other in none (Raji). The "recipient" cells were leukocytes of female infants; the cells were separated from 15 ml of peripheral blood after addition of 5 ml of a 6 percent dextran solution. The Burkitt cells were mixed immediately after irradiation or up to 6 days later with fresh or 1-day-old leukocyte suspensions (1 to 2 $\times$ 10^6 cells/ml, each) and incubated at 37°C for 2 to 24 hours in conical centrifuge tubes to permit close contact between the settled cells. Donor and recipient cells were handled separately in parallel, as controls. The mixed and individual cells were then seeded in equal numbers (2 to 4 $\times$ 10^6) onto monolayers of human diploid female fibroblasts (WI-38) which are known not to grow in suspension; these cultures were maintained by frequent renewal of the medium during a 2- to 8-week period. The media removed each time contained numerous floating cells which were used to set up suspension cultures, or to augment already existing ones of the corresponding series. These suspension cultures were observed for evidence of replication and eventual establishment of continuous lines.

Many facets of the culturing procedures remain to be analyzed. However, the available results are encouraging. In all seven experiments thus far, the normal peripheral leukocytes were induced to grow within 2 to 4 weeks after mixed cultivation with x-irradiated Jijoye cells (virus-positive); Raji cells (virus-negative) did not induce growth. Separate cultures of the leukocytes as well as of the x-irradiated Burkitt cells failed to survive for any length of time under our experimental conditions.

Five of the growing mixed cultures were maintained for 6 to 8 weeks before rapid replication began, after which subcultures could be made at 5- to 7-day intervals. Thus, sufficient numbers of cells for detailed analyses became available from these five cultures. From 100 to 300 cells in metaphase from each culture—Ba, Ry, McC, Na, and Re—were examined, and none contained a Y chromosome. Thus, certainly less than 0.3 to 1 percent of the cells, if any, could be of the donor type, and, at most, 0.03 to 0.05 percent of the total cell population would be expected to reveal immunofluorescence specific for the Burkitt-associated virus, since the Jijoye line contained from 3 to 5 percent stainable cells. Yet, when the five cultures were tested at