backcross generation mice resulting from crosses of A2G with A, and F_1, F_2 and first backcross generation mice resulting from crosses of A2G with C3H were challenged with neurotropic influenza A virus, strain NWS. The results were compatible with the hypothesis that a single dominant autosomal allele was responsible for resistance to the virus. The symbol *Mx* (myxovirus-resistant) is tentatively proposed for the allele involved.

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Photosensitization of Human Cell Cultures by Demethylchlortetracycline. (29293)

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Photosensitization following exposure to the sun when patients are being treated with demethylchlortetracycline (Declomycin) is well documented(1,2). Other tetracycline compounds produce this effect only rarely (3,4,5). No explanation for the differences in capacity of the different members of this group of antibiotics to produce this reaction is available, possibly because of lack of a laboratory method suitable for quantitative study of this problem. The present paper concerns a procedure applicable to the investigation of photosensitization and presents data which suggest a basis for the difference in activity of the various tetracyclines in inducing development of this phenomenon.

Materials and methods. Freshly-obtained human amnion was exposed to 0.5% trypsin dissolved in Hanks' balanced salt mixture for 2.5 hours. The cells were then washed and suspended in Eagle's medium to which horse serum (15%) was added. To aliquots containing 500,000 cells per ml, demethylchlortetracyline (DMCT), methacycline (MC), 6-methylene-5-hydroxytetracycline (supplied by Charles Pfizer & Co., Inc., New York) and tetracycline (TC) were added in concentrations ranging from 0.4 to 25 μg per ml. After incubation at room temperature for 18 hours, the treated cell suspensions were exposed to long and short UV light in the following manner: A 15-watt UV lamp (General Electric Co.) mounted in a box lined with aluminum foil was the source of the short wave light which was almost normochromic and had a peak wave length of 2537Å. A Woods light (Burton Mfg. Co., Los Angeles, Calif.) with a range of 3660Å to 3900Å was used to furnish the long wave UV. The cells being studied were placed in open Petri dishes at a distance of 20 inches from the light source and shaken mechanically at a rate of 140 per minute. The duration of exposure to short UV light was 10 seconds and to the long waves 30 and 120 seconds. At the end of these periods, the cells were placed in test tubes and plastic tissue culture flasks and incubated at 37° in a stationary position for 48 hours. At this point, the fluid was removed from all of the cultures and replaced with tetracycline-free Eagle's medium containing 100 units of penicillin and 50 μg of streytomycin per ml and 10% horse serum; these preparations were incubated at 37°C for 5 days when they were examined microscopically and photographed. Each experiment included a group of controls which were treated in a manner identical to the experimental cultures except that the cell suspensions were not exposed

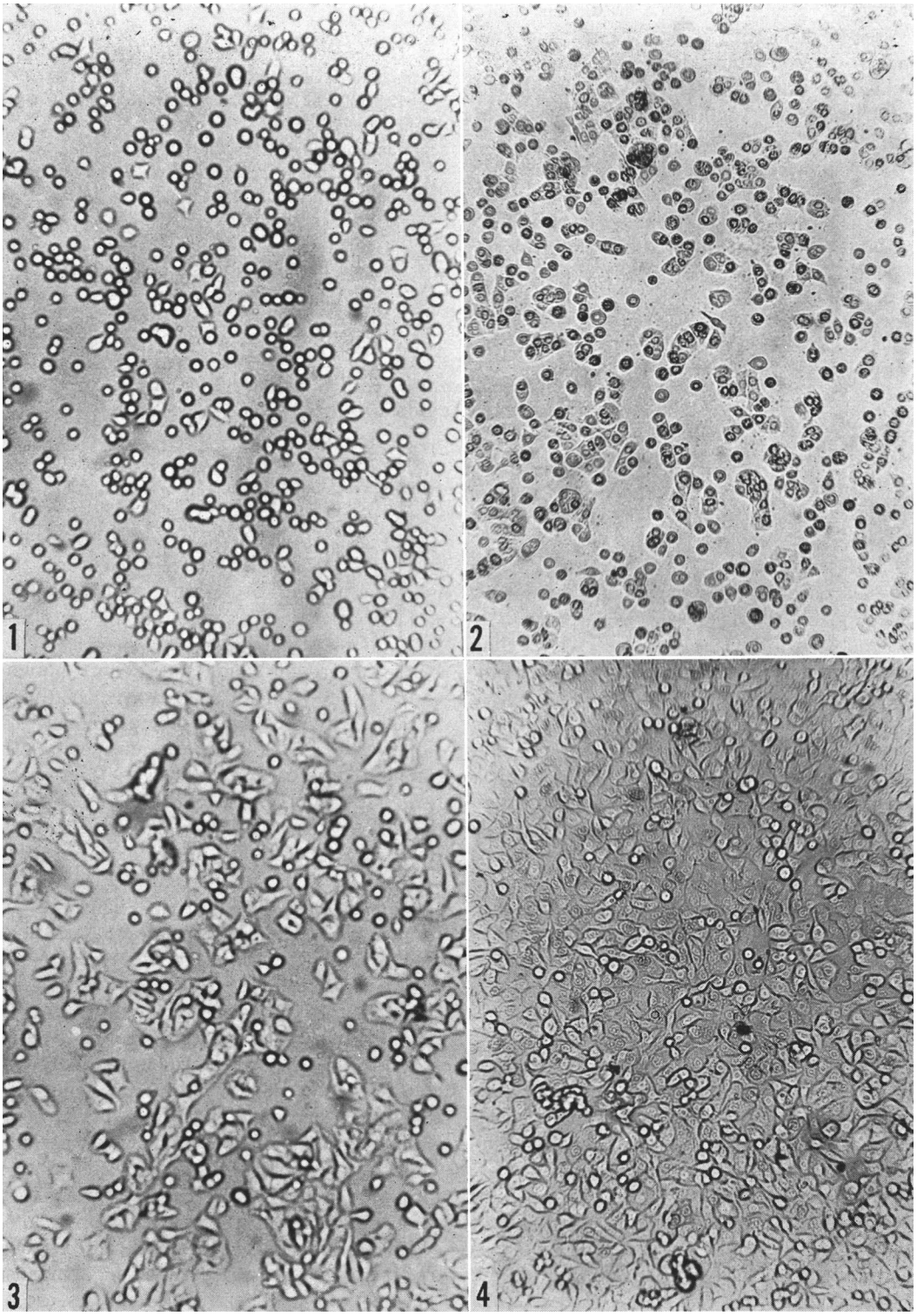

FIG. 1. 1+ Cell growth. FIG. 2. 2+ Cell growth.
FIG. 3. 3+ Cell growth. FIG. 4. 4+ Cell growth.

TABLE I. Effect of Tetracycline and Ultraviolet Irradiation on Growth of Human Amnion Cells.

		Cell growth			
		Drug concentration (μg/ml)			
	Drug	25	6.2	1.6	.4
Not irradiated	DMCT	3+	4+	4+	4+
	MC	3+	4+	4+	4+
	TC	4+	4+	4+	4+
	None	4+	—	—	—
Irradiated	DMCT	1+	1+	2+	3+
	MC	2+	3+	3+	3+
	TC	3+	3+	3+	3+
	None	3+	—	—	—

DMCT, demethylchlortetracycline; MC, methacycline; TC, tetracycline.

to ultraviolet light or not treated with one of the tetracycline compounds.

Results. The degree of growth present in control and treated tissue cultures at the end of the incubation was graded from 1+ to 4+ on the following bases: 1+, growth of about 10% of the cells (Fig. 1); 2+, growth of 50% of the cells (Fig. 2); 3+, growth of approximately 80% of the cells without formation of a cell sheet (Fig. 3); 4+, full growth and development of a sheet (Fig. 4).

The results of the studies in which short UV light (2537Å) was used are presented in Table I. Very little interference with cell growth was observed when the amnion cultures were treated with the tetracycline compounds alone. Both DMCT and MC produced slight inhibition of cell multiplication at a concentration of 25 μg per ml. Growth in the presence of TC was normal. Ultraviolet irradiation alone produced only a slight effect on cell cultures to which antibiotics had not been added. However, in the presence of 1.6, 6.2, and 25 μg per ml of DMCT, ultraviolet light treatment was followed by marked interference with growth of the tissue cultures. Cells treated with MC (25 μg per ml) were inhibited to a notable degree when subjected to irradiation; at lower concentrations, this drug was without effect. Cells treated with TC did not appear to be injured by UV light.

Exposure of cultures of human amnion cells to long wave UV light (3660Å to 3900Å) in the presence of the tetracycline compounds produced no changes. The degree of growth was the same as that in cells treated with the antibiotics and not subjected to irradiation. DMCT was exposed to UV light prior to addition to the tissue cultures in order to rule out the possibility that a change in the drug occurred due to irradiation; the effect of the irradiated antibiotic on cellular multiplication was no different than that of the untreated compound.

Discussion. Although both DMCT and MC, in a concentration of 25 μg per ml, inhibited the growth of cultures of human amnion to a slight degree, the exposure of cells treated with DMCT to short wave UV light produced pronounced inhibition of growth; quantities as small as 1.6 and 6.2 μg per ml interfered with cell multiplication. Cultures treated with MC and subjected to irradiation were affected to the same degree as those which were exposed to the drug alone. Exposure of DMCT to UV light prior to its addition to cell cultures did not increase its toxic effect. This suggests that the injurious effect of irradiation was dependent on changes produced in cells by the antibiotic and not on alterations which developed in the drug itself. These observations are consistent with the hypothesis that the observed phenomena resulted from photosensitization of cells by contact with DMCT.

Although photosensitization of human skin by drugs has been shown to be dependent on a number of factors(6), the length of the UV light to which exposure occurs is of great importance. DMCT has been found to be activated by light in the range of 2750Å to 3100Å. The spectrum most effective in producing sunburn, however, is 2900Å to 3100Å. Wave lengths shorter than 2900Å do not reach the earth from the sun because of the filtering action of the atmosphere. In addition, the quantity of energy required to produce erythema with light of 3200 to 4200Å is 500 to 800 times greater than with light of 3000Å; when wave length of 4200Å is used, the energy requirement is 1000-fold higher than when light of 3000Å is employed. These observations support the results obtained in the present study, since photosensitization of the tissue cultures in the presence

of DMCT was evident when short but not long wave UV light was used.

It is significant that photosensitization of cultured human cells was produced by concentrations of DMCT as low as 1.6 μg per ml. This is a level which is easily produced in the serum of patients to whom this drug is administered(7,8). The data suggest a possible explanation for the clinical observation that photosensitivity is a common untoward effect of administration of DMCT and is not a problem when MC or TC is used (1-5,7).

Summary. Exposure of cultured human amnion cells to some tetracycline compounds produced slight suppression of growth. When drug treated cells were subjected to irradiation with short-wave (2537Å) UV light, those to which demethylchlortetracycline had been added were severely damaged, the effect being moderate at a concentration of 1.6 μg per ml of the drug and marked at a level of 6.4 μg per ml. Methacycline and tetracycline were without significant effect. Exposure to long-wave (3960Å) UV light did not increase the slight damaging effects of the antibiotics on cells. The phenomenon was observed at concentrations of demethylchlortetracycline commonly present in the serum of patients treated with this agent and appears to be due to cellular injury produced by irradiation on cells which have been sensitized by exposure to drug.

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Autoradiographic Investigations of ^{14}C-labelled Thalidomide and Glutethimide in Pregnant Mice. (29294)

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Comprehensive investigations by Gross, Hoffmann, Keberle and Tripod(4) have demonstrated that numerous α-di-substituted glutarimides have a central nervous system depressing effect. Due to favorable pharmacological properties, the α-phenyl-α-ethyl-glutarimide was introduced in therapy as a hypnotic agent with the name of glutethimide.

By substituting nitrogen in the α-position of glutarimide, derivatives of glutamic acid are formed which possess essentially different chemical and pharmacological properties. Among them the compound N-phthalyl-d-l-glutamimide, known as thalidomide, showed central nervous system depressing action both in experimental animals (Kunz, Keller and Mückter(6) and in man. In large doses, however, it did not produce general anesthesia or lethal poisoning. Nor did the substance possess any anticonvulsive effect which is usually found associated with hypnotic agents. When introduced in therapy, thalidomide was therefore regarded as promising until the well-known toxic effects stopped its further use.

Recently the metabolic fate of glutethimide and thalidomide was investigated (Keberle, Hoffmann and Bernhard,(5); Faigle, Keberle, Riess and Schmid(3); Beckmann(1,2)). Glutethimide was found to be metabolized by oxidative processes, probably in the liver microsomes. Thalidomide however is transformed into various carbonic acids by hydrolytic reactions. As an attempt to elucidate

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