to obtain sufficient calcium to bind the proteins, it must have been generalized and took place in all of the bones. If we assume that *Sceloporus cyanogenys* has less than 2 ml of plasma, 4 mg of calcium could elevate the serum calcium levels to over 300 mg/100 ml. In the lizards used, the total skeleton weighed around 4 gm, and so the loss of only 0.1% of the skeletal weight could bring enough calcium into the circulating blood. Thus, the small amount of calcium removed from the lizard bones was difficult to observe histologically.

Previous investigators(3,9,11) interested in the role of estrogen in plasma protein synthesis have all considered that the liver was the site of origin. Our study has indicated that the "gamma globulin" component appears to be the main plasma protein that is increased in absolute quantity. Gamma globulin is synthesized in the reticuloendothelial system, particularly the plasma cells (2, 5, 6, 7), and estrogen causes not only an increased phagocytic index but also plasma cell hyperplasia and increased gamma globulin production(7). Therefore, it is suggested that in the lizard, estrogen induces an increased production of "gamma globulins" which may be produced by nonhepatic organs. Further studies will be conducted along these lines.

Summary. Adult *Sceloporus cyanogenys* were treated with 250 μg of estradiol valerate for 5, 11, 15 and 42 days. The bones were studied histologically and the plasma chemistry was determined. No morphological changes were observed in the bones. The plasma calcium and inorganic phosphorus were significantly elevated from the controls within 5 days after the injection. The proteins did not increase significantly until 11 days after injection, and the elevation occurred only in the "gamma globulin" component. It is suggested that estrogen induces increased "gamma globulin" synthesis whose origin is in part in the reticuloendothelial system.

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Immunization of Mice to Sarcoma 180 and Ehrlich Carcinoma with Ultraviolet-Killed Tumor Vaccine* (32609)

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As early as 1906 Ehrlich (1) reported that animals bearing a progressively growing tumor would reject a second transplant of the same tumor. Since that time it has become known that once a transplanted animal tumor is actively established, it may either enlarge progressively and kill the host, or upon reaching a certain point in development, may undergo spontaneous regression and spare the host (2–4). Animals with spontaneously regressed tumors were found to be immune to sub-

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sequent transplants of the same tumor (2,3). The incidence of this spontaneous regression is reported to be 5% for Sarcoma 180-bearing mice, 3% for Walker carcinoma 256, 52% for Novikoff Hepatoma, but not to occur in Ehrlich carcinoma (2).

During studies in our laboratory, we observed a low incidence of spontaneous regression of solid Sarcoma 180 as well as Ehrlich carcinoma in tumor bearing CF_1 white Swiss male mice. Upon challenging these surviving mice subcutaneously with living ascitic tumor of the homologous type, none of the mice developed solid tumors. In contrast, nontumor-bearing control mice which received the same challenge, all developed actively growing solid subcutaneous tumors. Studies were then undertaken to determine whether immunity to Sarcoma 180 and Ehrlich carcinoma could be induced artificially in mice with use of ultraviolet-irradiated, killed neoplastic vaccine.

The first trial of ultraviolet irradiation in tumor research was not for vaccine production but for its possible therapeutic action in rat and mouse carcinoma (5). Although irradiation reduced proliferative activity of tumor implants, whole body irradiation of tumor bearing animals failed to exert an effect on tumor growth. The first attempt to use ultraviolet irradiation for cell vaccine production was by Cosby *et al.* (6) in 1962, who reported incomplete protection against challenge with living tumor in AKR mice which had been immunized with ultraviolet-irradiated and attenuated but living BW5147 lymphatic leukemia cells.

Material and Methods. Tumor maintenance. Sarcoma 180 and Ehrlich carcinoma were contributed by Sloan Kettering Institute for Cancer Research (Dr. K. Sugiura). The ascitic forms of both tumors were maintained in 6-week-old 20 gm CF_1 white Swiss male mice (Carworth Farms, Inc.) by weekly intraperitoneal injections per mouse of 0.2 ml undiluted ascitic fluid. Solid forms of both tumors were produced by the subcutaneous injection in the right axillary region of 3 million ascitic cells contained within 0.2 ml saline. Adenocarcinoma 755 was contributed by Microbiological Associates, Inc. and was maintained in 6-week-old, male inbred C57 BL/6J mice (The Jackson Laboratory) by subcutaneous trocar implantation every 12-14 days. All mice were housed in stainless steel cages with sawdust bedding in air conditioned quarters and all received standard mouse pellet (Rockland Farms, Inc.) and water *ad libitum.*

Preparation of ultraviolet-irradiated neoplastic cell vaccines. Actively growing Sarcoma 180 and Ehrlich carcinoma tumor cells were obtained by syringe withdrawal of ascitic fluid from the abdomens of CF_1 mice 7 days after prior intraperitoneal injection of living tumor cells. This tumor-containing ascitic fluid was diluted with sterile 0.85% saline to produce a count of 3 million tumor cells per 0.2 ml. The tumorigenicity of the cells was destroyed by exposing 2 ml aliquots of tumor cell suspension (one part undiluted ascites to 8–15 parts saline) to ultraviolet irradiation at 2537 Å in air at room temperature at 1 cm distance in open 55 × 55 mm sterile aluminum dishes with frequent gentle agitation. The depth of the suspension was approximately 1 mm. Periods of irradiation were 2, 5, 10, and 15 min with a Mineralight (Ultra Violet Prod., Inc., San Gabriel, California). Irradiated tumor cell suspensions were used promptly for immunization and were not stored. The immunization schedule in mice consisted of subcutaneous or intraperitoneal injections of 3 million tumor cells each contained within 0.2 ml volume and given weekly for 3 weeks. Following a 2-week rest period, the mice were each challenged with 3 million living ascitic 7-day-old tumor cells of the homologous type by direct subcutaneous inoculation in the right axilla. The effect of ultraviolet irradiation on the viability of the tumor cells was evaluated by (a) their uptake of supravital stain after 15 min contact with 0.04% aqueous solution of Janus green, (b) by their tumorigenicity in mice and (c) their oxygen uptake.

In order to determine the separate immunizing capacities of the cellular and acellular fractions of Sarcoma 180 ascitic fluid, these components were first separated by means of ordinary centrifugation at 3000 rpm. The clear acellular supernatant fluid was diluted with saline in the same proportion as whole cellular ascitic fluid after which it was

irradiated with ultraviolet at 1 cm distance in 2 ml aliquots in air at room temperature with gentle agitation for 10 min. Fresh tumor cells which remained in the sediment were resuspended in an equal volume of sterile saline and washed three times after alternating centrifugation and resuspension. The washed cell suspension contained 3 million living tumor cells per 0.2 ml and was irradiated with ultraviolet, as above. An aliquot containing a similar number of washed living tumor cells was completely disrupted at —20°C in a Hughes Press at 10,000 lbs per sq inch and the smashed dead cells were irradiated with ultraviolet for 10 min. Other aliquots of washed, intact, nonirradiated living tumor cells, each containing 3 million cells per 0.2 ml were separately killed and preserved with 0.5% formaldehyde and 1:1000 merthiolate. The immunization schedule of CF_1 white Swiss male mice consisted of intraperitoneal injections once weekly for 3 weeks of the different cellular and acellular ascitic fluid fractions. Each injection dose contained either 3 million killed whole cells per 0.2 ml, disrupted tumor cells or the equivalent volume of acellular supernatant fluid. Two weeks after the last immunization, all mice including the non-immunized control groups, received direct subcutaneous challenge with 3 million 7-day-old living Sarcoma 180 cells each contained within a volume of 0.2 ml.

The effects of ultraviolet irradiation on consumption of oxygen by 7-day-old Sarcoma 180 ascitic tumor cells was measured in a Warburg respirometer at 37°C. Five ml aliquots of tumor cells in saline at pH 6.6 containing 75 million cells were incubated in manometer flasks following 10 min irradiation of one of the suspensions. The center wells contained 0.5 ml of 10% KOH to absorb CO_2. Ultraviolet irradiation caused a rise in pH of the tumor suspension from 6.6 to 7.3. Oxygen consumption was measured and expressed as cumulative μliter of O_2 uptake. The first reading was determined after a 20-min period of equilibration.

Results. Effects of ultraviolet irradiation on Sarcoma 180 and Ehrlich carcinoma. The lethal effects of ultraviolet irradiation on whole ascites and washed tumor cells of Sarcoma 180 and Ehrlich carcinoma was found to depend upon (a) the duration of irradiation at 2537 Å (b) the distance between the source of the irradiation and the cell suspension and (c) the density of the suspension. Irradiation for 2 or 5 min failed to kill all of the tumor cells and they continued to absorb Janus green, as observed microscopically, as well as to produce growing tumors on intraperitoneal implantation in mice. However, after 10 or 15-min irradiation, the cells lost their ability to absorb the vital stain and also lost their ability to produce either ascitic or solid tumors in mice by intraperitoneal or subcutaneous routes respectively. The majority of cells remained physically intact after irradiation (Fig. 1), however all of them showed marked swelling and ballooning of the cytoplasm, attributed to an increase in cell membrane permeability. Perinuclear agglomeration of cytoplasmic granules was also observed (Fig. 1). Nonirradiated 7-day-old Sarcoma 180 ascitic tumor cells freshly withdrawn from the abdomen had average measurements of 14.2 × 15.9 μ, while after 10 min irradiation cell dimensions had almost doubled, *i.e.*, 24.1 × 30.6 μ (Fig. 1). Nonirradiated Sarcoma 180 ascitic tumor cells of the same age showed an active oxygen uptake at 37°C in the Warburg respirometer, while cells irradiated for 10 min showed no oxygen uptake whatsoever over a 2-hour period with or without addition of dextrose (Fig. 2). Ten min was then selected as the standard time for ultraviolet irradiation required for the production of a nonviable, nontumorigenic cell suspension.

Effects of variation in the route of immunization and challenge on tumor immunity. Weekly injections for 3 weeks of freshly killed ultraviolet-irradiated tumor cells of Sarcoma 180 and Ehrlich carcinoma caused mice to become significantly resistant to subsequent challenge with living tumor cells of the same type (Table I). In contrast, all of the nonimmunized mice that were challenged in the same manner developed solid subcutaneous tumors and all died. Immunization *via* the intraperitoneal route was noted to produce better protection to challenge with living Sarcoma 180 than the subcutaneous route. The former route was selected as the standard for routine immunization in subsequent ex-

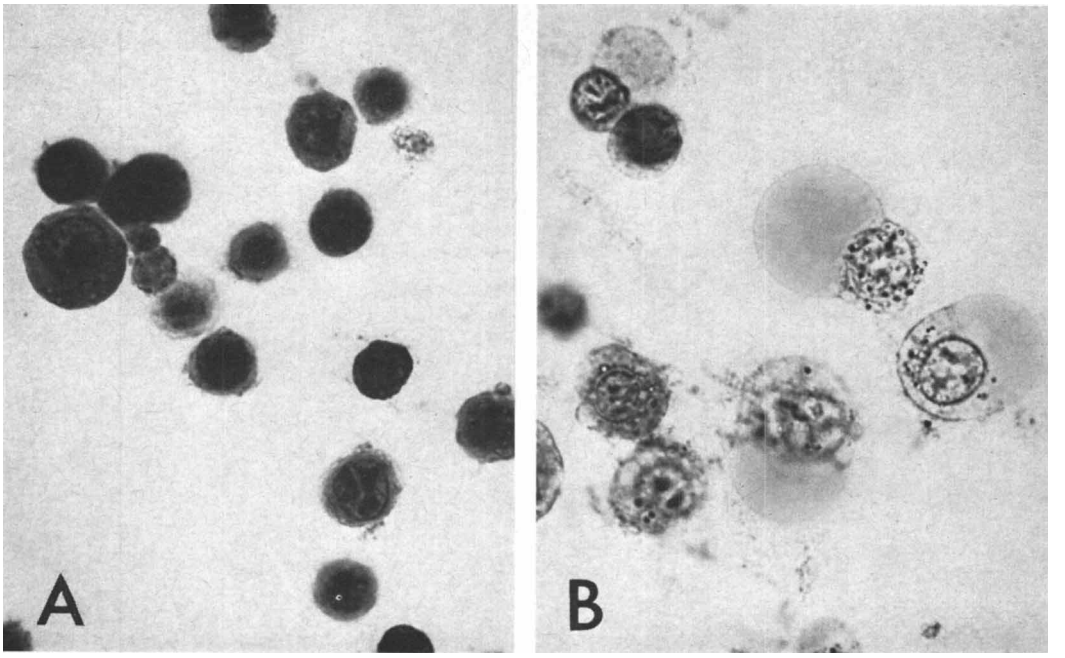

FIG. 1. Cytological effect of ultraviolet irradiation *in vitro* on 7-day-old ascitic Sarcoma 180 cells. A. Living nonirradiated ascitic tumor cells show active uptake of Janus green (×600). B. Tumor cells irradiated for 10 min with ultraviolet light at 2537 Å show poor or absent uptake of Janus green, ballooning of cytoplasma and perinuclear aggregation of cytoplasmic granules, indicative of cell death (×600).

periments. The standard challenge test was the subcutaneous injection of 3 million living tumor cells given 14 days after the last immunization.

Spleens of Sarcoma 180-immunized mice had an average weight of 0.14 gm and measured 4.6 × 17.6 mm at the end of the test period, while those of the nonimmunized and tumor bearing mice were much larger, averaging 0.33 gm and measuring 7.2 × 24.4 mm.

Duration of immunity. Mice immunized either intraperitoneally or subcutaneously with three weekly injections of ultraviolet-irradiated, killed Sarcoma 180 or Ehrlich carcinoma cells received a single subcutaneous challenge with 3 million living tumor cells of the homologous tumor at 15, 60, and 120 days after the last immunization. As illustrated

TABLE I. Effect of Routes of Immunization and Challenge on Immunity to Sarcoma 180 and Ehrlich Carcinoma in CF_1 White Swiss Male Mice.

Exptl. group	Tumor type	Route of immunization with 3×10^6 UV-killed cells[a]	Route of challenge with 3×10^6 living cells[b]	No. recipient mice developing solid tumor/no. tested		Mortality (%)	
				Immunized	Non-immunized	Immunized	Non-immunized
A	Sarcoma 180	Subcutaneous	Subcutaneous	1/9	5/5	11	100
B	Sarcoma 180	Intraperitoneal	Intraperitoneal	1/9	10/10	11	100
C	Sarcoma 180	Intraperitoneal	Subcutaneous	0/10	10/10	0	100
D	Ehrlich carcinoma	Intraperitoneal	Subcutaneous	0/5	5/5	0	100
E	Ehrlich carcinoma	Subcutaneous	Intraperitoneal	0/5	5/5	0	100

[a] 3 weekly injections.

[b] 2 weeks after last immunization.

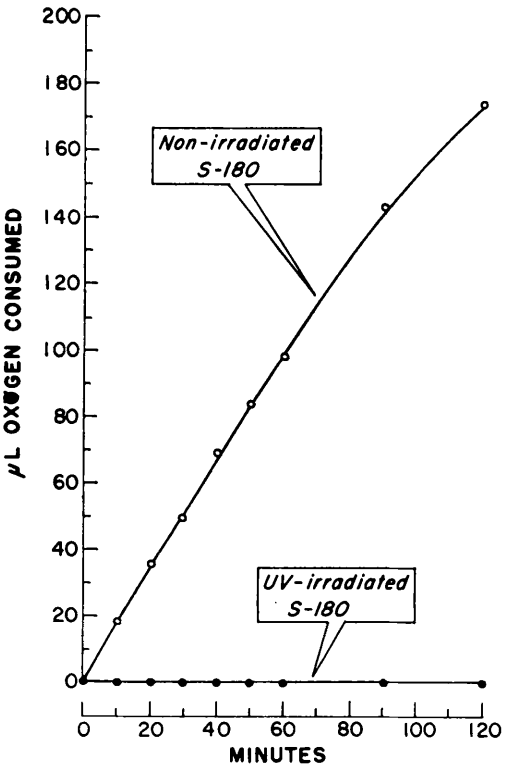

FIG. 2. Respiration *in vitro* of ultraviolet-irradiated and nonirradiated freshly drawn 7-day-old ascitic Sarcoma 180 cells. Nonirradiated cells show active uptake of oxygen, while irradiated cells do not respire. Manometer flasks at 37°C contain 75 × 10^5 cells/5 ml saline suspension. Center well charged with 0.5 ml 10% KOH. Following a 20 min period of equilibration, oxygen consumption was measured and expressed as cumulative μliters of oxygen uptake. Ultraviolet-irradiated cells received 10 min irradiation at 2537 Å at 1 cm distance in 1 mm thick film at room temperature with resulting increase in pH from 6.6 to 7.3.

in Table II, the majority of mice were still immune to challenge 4 months after the last immunization while nonimmunized mice of the same age and lot number showed no protection against the challenge dose of either tumor. Greater protection was again produced by the intraperitoneal rather than the subcutaneous route.

The marked difference between intraperitoneally immunized and nonimmunized mice in their responses to single challenges with living Sarcoma 180 tumor cells is seen in Figs. 3 and 4. Soon after challenge all of the 20-nonimmunized mice developed rapidly growing, large, subcutaneous tumors which produced 80% mortality within 40 days and 100% in 75 days. In contrast, all of the 14-immunized mice showed only tiny, soft subcutaneous masses after challenge which did not enlarge and which gradually disappeared with time. While 2 of the immunized mice died during the observation period of causes unrelated to tumor bearing, the remaining 12 of the 14 immunized mice were alive and healthy and were without sign of tumor eight months after the initial challenge (Fig. 4).

Titration of the immune state. The site of challenge and the number of living tumor cells in each challenge dose did have an effect on the demonstration of immunity in tumor-immunized mice. It was found possible to exceed the immune state produced by three injections of ultraviolet-irradiated killed neoplastic cell vaccine with the use of an excessively large challenge dose of living tumor cells. Thus, all of the mice immunized intraperitoneally with 3 million killed tumor cells per dose were solidly resistant to direct subcutaneous challenge with the same number of living homologous tumor cells (Table III). However, when the homologous challenge dose was increased to 30 million cells, resistance to challenge decreased to 80% for Sarcoma

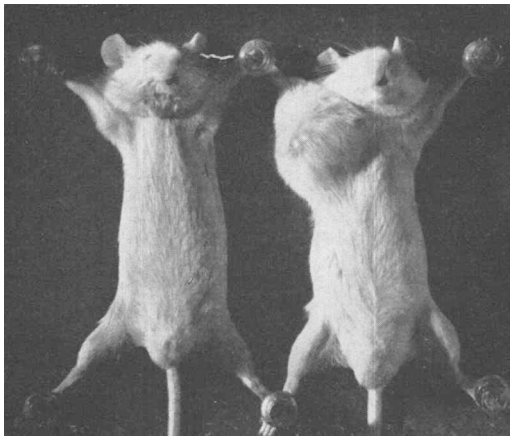

FIG. 3. Response of CF_1 male Swiss mice 21 days after direct subcutaneous challenge with 3 × 10^6 living Sarcoma 180 ascitic cells. Left: Mouse immunized with 3 weekly ip injections of 3 × 10^6 ultraviolet-irradiated killed Sarcoma 180 ascitic cells. Right: Nonimmunized mouse.

TABLE II. Effect of Route of Immunization and Challenge on Duration of Immunity in CF_1 Mice. Mice immunized 3 times with 3×10^6 UV irradiated ascitic tumor cells and challenged once with 3×10^6 living cells at 15, 60 and 120 days after immunization.

Tumor type	Route of immunization and challenge	No. recipient mice developing solid tumor/no. tested					
		Immunized			Nonimmunized		
		15 days	60 days	120 days	15 days	60 days	120 days
Sarcoma 180	ip immunization sc challenge	1/10	0/10	0/15	10/10	10/10	9/9
	ip immunization ip challenge	0/3	0/10	—	3/3	10/10	—
	sc immunization sc challenge	0/5	—	1/9	5/5	—	9/9
Ehrlich carcinoma	ip immunization sc challenge	2/10	1/10	—	10/10	10/10	—
	ip immunization ip challenge	0/10	2/5	—	10/10	10/10	—
	sc immunization sc challenge	—	—	2/8	—	—	10/10
	sc immunization ip challenge	0/5	—	—	—	—	—
	Total	3/43	3/35	3/32	38/38	40/40	28/28

180-immunized mice and to 57% for Ehrlich carcinoma-immunized mice. Less protective effect was observed when the challenge dose of living tumor cells was given intraperitoneally rather than subcutaneously (Table III). When the former route was employed for challenge 100% of the Sarcoma 180-immunized mice resisted a single challenge of 300,000 living cells, 67% resisted challenge with 3 million cells but none resisted challenge with 30 million living tumor cells. In contrast, nonimmunized mice showed no resistance to challenge with any of these doses by either route.

Immunological specificity. Mice immunized intraperitoneally with ultraviolet-killed, Sarcoma 180 vaccine were not only immune to challenge with living tumor cells of the homologous type, but were also found to be cross immune to living Ehrlich carcinoma tumor cells (Table IV). Thus, all of the Sarcoma 180-immunized mice resisted challenge with 300,000 living Ehrlich carcinoma cells, 80% resisted challenge with 3 million cells and 40% of the mice resisted 30 million living cells. On the other hand, nonimmunized mice were uniformly susceptible to all three levels of challenge of Ehrlich carcinoma. A reverse experiment was not performed in which Ehrlich carcinoma-immunized mice were challenged with living Sarcoma 180. This and numerous other aspects of the specificity of tumor immunity warrants further investigation.

The normal susceptibility of C57 BL/6J mice to adenocarcinoma 755 was not affected by prior immunization with 3 weekly intraperitoneal injections of ultraviolet-killed Sarcoma 180 ascitic tumor cells.

Cell mediated nature of the immunity produced by ultraviolet-irradiated tumor cell vaccine. In the next experiment, the majority (84%) of mice immunized with ultraviolet-irradiated, killed, whole ascitic Sarcoma 180 tumor fluid were rendered immune to direct subcutaneous challenge with 3 million living homologous tumor cells (Table V). When the tumor cells were washed prior to ultraviolet irradiation and then used for immunization, 68% of the mice were still immune to challenge. However, disruption of these washed cells at —20°C in a Hughes press, prior to their irradiation with ultraviolet, all but eliminated this immunogenic property (Table V). Mice immunized with acellular super-

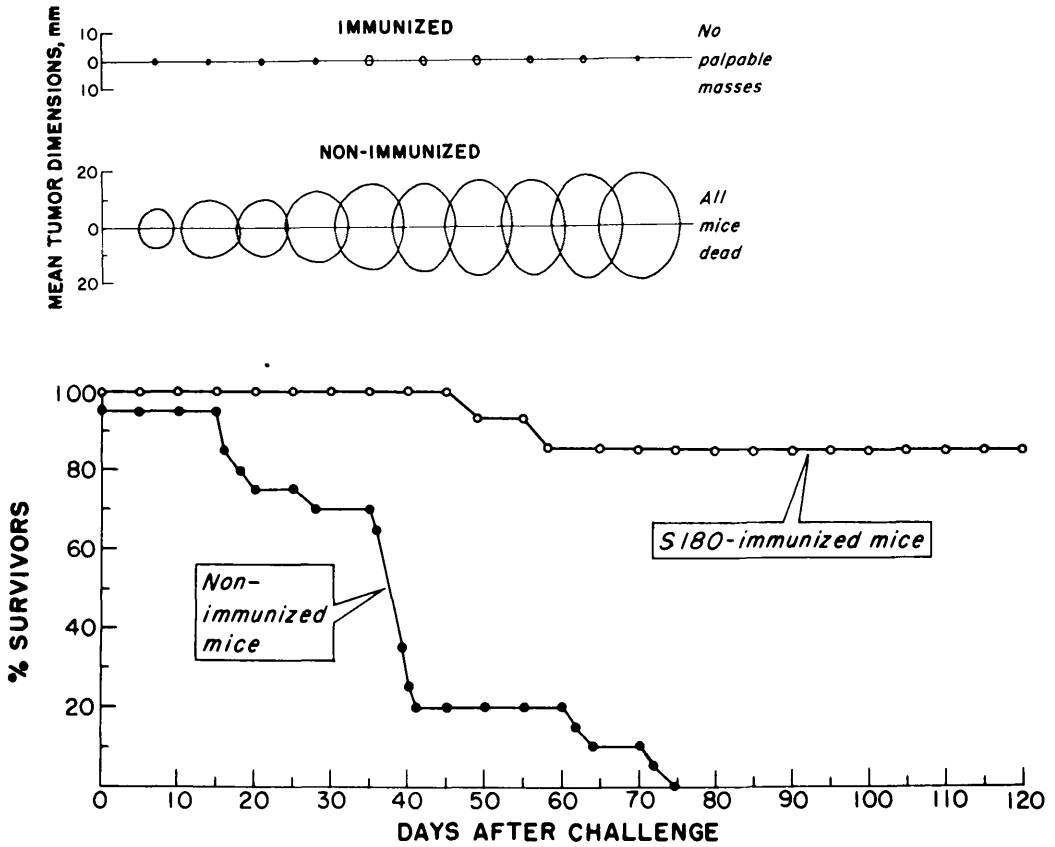

FIG. 4. Comparative tumor growth and mortality of 14-intraperitoneally-immunized and 20-nonimmunized CF_1 white Swiss male mice following single subcutaneous challenge with 3×10^6 living ascitic Sarcoma 180 cells. Prior immunization consisted of 3 weekly injections of 3×10^6 ultraviolet-irradiated killed ascitic Sarcoma 180 cells.

natant fluid derived from the ascitic tumor fluid, also showed no immunity to challenge. Formaldehyde-killed and merthiolate-killed washed intact tumor cells were likewise nonimmunogenic and nonprotective (Table V). These results would indicate that immunity produced in mice by injection of ultraviolet-irradiated, killed, ascitic Sarcoma 180 lies in the use of *intact,* freshly killed, irradiated tumor cells rather than disrupted cell fragments or cell extracts. The immunity appears to be cell mediated leading to the inference that the host has provided an anticellular, nonhumoral factor responsible for the development of immunity to tumor challenge.

Passive transfer of serum of Sarcoma 180-immunized mice at a dose of 0.4 ml serum per mouse failed in several instances to protect nonimmunized mice from direct subcutaneous challenge with living homologous tumor cells and failed to modify the subsequent development of the tumors that were produced. Serum from Sarcoma 180-immunized mice showed no demonstrable precipitins against whole ascitic tumor fluid, acellular supernatant fluid, washed living ascitic tumor cells or disrupted cells. This was determined by the Ouchterlony technique with sodium barbital HCl buffer and 1% agar at pH 8.2. Agglutinins could not be demonstrated in the serum using either slide or tube agglutination techniques.

Discussion. Numerous procedures have been employed in the past for attenuating tumor cells for immunizing purposes including X-ray, light and dyes, nitrogen mustard and its oxide, nitrous acid, formaldehyde, lyophilization and ^{60}Co-ray irradiation(7). In most instances varying and incomplete protection

was produced. As far as can be ascertained from the literature, the present report is the first in which a killed, nontumorigenic irradiated cell vaccine is shown to produce a high degree of immunity in mice persisting for many months after immunization. The immunogenicity of the irradiated tumor cell vaccines appear to be wholly dependent upon their prior exposure to nonionizing ultraviolet irradiation. Treatment of the same cells with formaldehyde or merthiolate rendered them incapable of mitosis and physically intact but nonimmunogenic.

It is known that destruction by ultraviolet irradiation of the replicative functions of bacteria and viruses, and presumably other living cells, is dependent upon the wavelength of the light employed. The lethal effect of

TABLE III. Degree of Immunity Produced in CF_1 Mice by Weekly ip Injections of 3×10^6 UV Irradiated Killed Tumor Cells Followed by Challenge 14 Days Later with Varying Doses of Living Homologous Tumor Cells.

Tumor type	Route of challenge		Challenge dose of living tumor cells					
			Immunized mice			Nonimmunized mice		
			3×10^7	3×10^6	3×10^5	3×10^7	3×10^6	3×10^5
Sarcoma 180	Subcutaneous	Solid tumor takes	2/10	0/10	0/10	10/10	10/10	3/10
		Mice immune (%)	80	100	100	0	0	0
		Mean tumor weight (gm)	0.97	0	0	2.87	2.41	1.31
		Mortality 14 days after challenge	0/10	0/10	0/9	1/9	1/9	0/10
	Intraperitoneal	Tumor takes (ascites)	9/9	3/9	0/10	9/9	10/10	10/10
		Mice immune (%)	0	67	100	0	0	0
		Mortality 14 days after challenge	6/9	1/9	0/10	1/9	0/10	0/10
Ehrlich carcinoma	Subcutaneous	Solid tumor takes	3/7	1/7	0/7	10/10	10/10	10/10
		Mice immune (%)	57	86	100	0	0	0
		Mean tumor weight (gm)	1.08	0.41	0	1.89	2.1	1.58
		Mortality 14 days after challenge	0/7	0/7	0/7	0/10	1/10	2/10

TABLE IV. Cross Immunity of Sarcoma 180-Immune CF_1 Mice[a] to Ehrlich Carcinoma.

	Challenge dose of living Ehrlich carcinoma cells					
	Immunized mice			Nonimmunized mice		
	3×10^7[b]	3×10^6	3×10^5	3×10^7	3×10^6	3×10^5
Solid tumor takes	9/15	3/15	0/15	15/15	15/15	15/15
Mice immune (%)	40	80	100	0	0	0
Mean tumor weight (gm)	2.99	0.51	0	4.61	4.36	3.15
Mortality 14 days after challenge	5/15	1/15	0/15	7/15	2/15	2/15

[a] Mice immunized first with 3 weekly ip injections of 3×10^6 UV killed S-180 tumor cells, followed 14 days later by sc challenge.

[b] Undiluted ascites tumor.

TABLE V. Comparative Immunizing Effect of Sarcoma 180 Ascites Fractions.[a]

	Immunized mice						
	Whole ascitic tumor fluid	Acellular supernatant fluid	Washed intact tumor cells	Washed disrupted tumor cells (Hughes press)	Formalin (0.5%) killed intact tumor cells	Merthiolate (1:1000) killed intact tumor cells	Nonimmunized mice
UV irradiation of vaccine prior to immunization	+	+	+	+	—	—	
Solid tumor takes	3/19	20/20	6/19	19/20	9/9	10/10	20/20
Mice immune (%)	84	0	68	5	0	0	0
Mean tumor weight (gm)	0.75	3.30	0.97	3.19	3.13	3.15	3.54
Mortality 14 days after challenge	0/19	3/20	1/20	0/20	1/9	2/10	3/20

[a] Mice immunized first with 3 weekly ip injections of 3×10^6 treated S-180 tumor cells followed 14 days later by sc challenge with 3×10^6 living cells or ascites fractions.

ultraviolet irradiation is greatest at a wavelength just below 2600 Å which is also the wavelength of the maximum absorption by purine and pyrimidine bases in DNA and RNA(8). As is to be expected from their content of purines and pyrimidines, intact nucleic acids show the greatest selective absorption in the range 2500 to 2600Å which is the range of the standard germicidal ultraviolet lamp that emits most of its energy at 2540 Å and which was used in these studies.

The effects of ultraviolet irradiation on nucleic acids and their constituents have been the subject of numerous studies(9,10). At the dose of radiation in the range responsible for biological effects, no measurable changes have been reported in the intrinsic viscosity of nucleic acids, their molecular weight or integrity of internucleotide linkages(9). Carbohydrate moieties are also unaffected.

The damage accompanying biological inactivation is thought to be highly localized in character(9). Ultraviolet damage to DNA includes photohydration, cross-linking of strands and formation of dimers between adjacent thymine residues(11,12). Ultraviolet irradiation partially or completely prevents DNA replication by causing covalent bonds to form between thymine residues in the DNA chain. Thymine dimerization may account for a large part of the ultraviolet-induced damage to transforming activity in *Hemophilus influenzae*(12) as well as loss of DNA synthesis in *E. coli*(13). It is also known to cause uracil dimerization and to inactivate transfer RNA function in *E. coli*(14). In addition to its effects on DNA, ultraviolet light at 2530 Å breaks the cytoplasmic membrane of *Candida utilis*(15). Irradiation at 2537 Å has been shown to destroy activity of enzymes such as ribonuclease by disrupting specific cystines in the enzyme(16). Also, a large part of the most intense incident ultraviolet radiation around 2540 Å would be absorbed by cytoplasmic nucleic acids, which are concerned with protein synthesis(17).

Ultraviolet irradiation appears to interrupt replicative, respiratory and metabolic functions of Sarcoma 180 and Ehrlich carcinoma suspensions and render them nontumorigenic. However, the cells remain physically intact and are immunogenic. Al-

though irradiation did not appear to rupture the cytoplasmic membranes of Sarcoma 180 or Ehrlich carcinoma cells, it did exert a profound effect on membrane permeability (Fig. 1). Studies of the precise effect of irradiation on the cell membrane by electron micrography would be of interest.

We observed that Sarcoma 180 tumor cell vaccine produced by treatment with formaldehyde, merthiolate or pressure cell disruption afforded no protection against subsequent challenge with homologous cells. In contrast, ultraviolet-irradiated, killed washed tumor cells or the irradiated whole ascitic tumor fluid gave nearly complete protection. The absence of antibodies in the tumor immunized mice, the failure of passive transfer of immunity and the complete loss of immunogenicity of the vaccine by cell disruption, leads to the conclusion that the immunity produced by ultraviolet-irradiated tumor cell vaccine is cell mediated. It suggests the development in the immunized host of a learned nonhumoral immune response oriented towards destruction of the living tumor cell, which quite possibly may be due to immune mononuclear cells.

The destruction of target cells by immune mononuclear cells has been demonstrated recently both *in vitro* and *in vivo* experiments (18–20). Bennett (20) has shown that peritoneal macrophages, lymphocytes and lymph node cells derived from C57BL mice immunized with EL4 ascites tumor were capable of suppressing the growth *in vivo* of the same tumor. Rosenau and Moon(18) demonstrated lysis of tumor cells in tissue culture by sensitized lymphocytes in the absence of antibodies or complement. The lytic effect was attributed to modification of the functional capacity of lymphocytes and not to absorbed cytotoxic antibodies(19). The tumor suppressive activity of macrophages derived from immunized mice was considered to be an expression of cell-associated immunity(20). Also, Coggin *et al.*(21) reported cell mediated immunity in the hamster that received SV_{40}-virus induced tumor which had been inactivated with gamma irradiation.

The fact that the peritoneal route of immunization was superior to the subcutaneous route with both Sarcoma 180 and Ehrlich carcinoma in these studies fits the concept of the emergence of immune mononuclear cells, since there would be a much greater outpouring of these cells in the peritoneal cavity than in a subcutaneous site. Further research is warranted to confirm the true nature of the immunity produced, to ascertain whether ascitic or neoplastic tissue cultures of other tumor systems lend themselves to effective vaccine production and to uncover other phenomena related to control of tumor in man and animals.

Exposure to nonionizing ultraviolet irradiation results in effects different from those of ionizing radiation. Ultraviolet irradiation of Sarcoma 180 and Ehrlich carcinoma caused perinuclear agglomeration of cytoplasmic granules and produced swelling and ballooning of the cytoplasm indicating an increase in cell membrane permeability. On the other hand, X-irradiation of Ehrlich carcinoma was reported to show alterations of the nucleus only (22). Although these cytological differences could be due merely to degree of exposure to radiation, they more likely represent differences in specific damage to the cell apparatus, which injuries would be more discernible by electron micrography. In addition, the level of immunity produced in mice with tumor cell vaccine prepared with nonionizing ultraviolet irradiation is considerably greater than that produced with ionizing X-irradiation. Contamin(23) was the first to produce partial immunity in mice with X-irradiated tumor cells. Later, limited and incomplete immunity was reported in mice against Sarcoma 180 and Ehrlich carcinoma with the use of X-irradiated tumor cell vaccine(22,24–27).

Summary. A high level of immunity of long duration was produced in CF_1 white Swiss mice against Sarcoma 180 and Ehrlich carcinoma by 3 weekly injections of homologous tumor cells killed by ultraviolet irradiation at 2537 Å. Intraperitoneal route of immunization afforded more protection than the subcutaneous route. Significant immunity to challenge with living Sarcoma 180 cells was still present eight months after immunization. Sarcoma 180-immunized mice were also cross immune to Ehrlich carcinoma. Passive transfer of serum from Sarcoma 180-immunized mice did not protect nonimmunized mice. Neither precipitins nor agglutinins could be demonstrated

in the sera of the immunized mice against homologous intact or disrupted tumor cells or cell supernates. The loss of immunogenicity of tumor cell vaccine upon cell disruption and the absence of humoral antibodies and passive transfer leads to the conclusion that immunity produced by irradiated, killed tumor cell vaccine is primarily cell mediated.

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The Effect of Lactate Infusion on Serum Uric Acid* (32610)

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In 1923 Gibson and Doisy(1) showed that the ingestion of sodium lactate was associated with decreased renal excretion of uric acid and a slight elevation of blood uric acid. Clausen (2) identified lactic acidosis two years later in a group of children with dehydration and circulatory insufficiency. Both observations remained relatively obscure till the present time.

In recent years there has been intensive interest in studies related to blood lactate, per se, due to the recognition of spontaneous lactic acidosis by Huckabee(3,4). Studies by Yü *et al.* (5) have generated much interest in the relationship of blood lactate to the renal

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