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ULTRAVIOLET LIGHT INDUCED CONNECTIVE TISSUE CHANGES IN RAT SKIN: A HISTOPATHOLOGIC AND HISTOCHEMICAL STUDY*

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It is generally accepted that chronic exposure to sunlight results in solar elastosis of human skin. However, experimental production of cutaneous elastotic changes in animals using artificial ultraviolet light has only rarely been reported. Utilizing histochemical methods, Sams, Smith and Burk demonstrated focal dermal elastosis in mice following prolonged exposure to artificial ultraviolet light (1).

The purpose of this paper is to record our observations on ultraviolet light induced connective tissue changes in rat skin.

MATERIALS AND METHODS

The animals used for this investigation were Fischer strain 344 albino female rats weighing approximately 100 grams at the onset of the experiment. Thirty animals were shaved on the dorsum with electric clippers weekly and exposed to ultraviolet light (UVL) three days a week for a total period of 27 weeks. Fifteen animals were used as controls and were shaved weekly but were not exposed to UVL. The exposure to UVL was for 20 minutes at a distance of 62 centimeters from a bank of 4 Westinghouse FS 40 fluorescent sun lamps. Biopsies from the dorsum of 4 treated animals and 2 control animals were taken after one week of UVL exposure and subsequently at 2 week intervals throughout the 27 week period of light exposure and afterward for a total period of 46 weeks. At 46 weeks, all of the UVL treated animals and all control animals were sacrificed for additional histopathologic and histochemical studies.

One-half of each tissue specimen was frozen on solid carbon dioxide and the remainder was embedded in paraffin. Fifty microns cryostat sections were processed for demonstration of blood vessels using the alkaline phosphatase technique (2), and 12 microns cryostat sections were used for demonstration of elastic tissue with the aldehyde-fuchsin stain at pH 1.7 (3). The following procedures were performed on paraffin embedded tissue sections from each specimen: hematoxylin and eosin; periodic acid-Schiff (PAS) reaction with and without diastase digestion; colloidal iron reaction with and without bovine testicular hyaluronidase digestion for one hour at 37 C (3); alcian blue stain at pH 2.5 and 0.4 (3); aldehyde-fuchsin stain at pH 1.7 at 0.4 (3); Movat's pentachrome I stain (4); and Snook's reticulum stain (5).

RESULTS

Macroscopic Changes. The animals exposed to UVL showed a mild diffuse erythema on the dorsum after one week. After two weeks the ears showed mild erythema and telangiectasia, and this change continued during the period of exposure. Most of the animals showed severe erythema, scaling, and crusting of the dorsum by five weeks and gross changes progressed to include ulcerations and alopecia of varying degrees from 9 weeks until UVL was discontinued at 27 weeks. The skin began to heal after UVL was stopped but some scarring and mild erythema remained. Two animals developed a papilloma on UVL exposed sites on the back of the ears. No abnormal changes were seen in the control animals.

Histopathologic and Histochemical Observations. The superficial corium showed mild to moderate interstitial edema and a minimal perivascular cellular infiltration after three weeks of UVL exposure. An increased number of lymphocytes and histiocytes were seen at 5 weeks, and after 7 weeks was more prominent and the cellular infiltrate included polymorphonuclear leukocytes, fibrocytes and mast cells. Cellular infiltration and edema in the superficial corium continued to increase until the 19th week after which it remained relatively constant up to 27 weeks when exposure to UVL was stopped. After 27 weeks, fibrocytic proliferation became pronounced. Edema and inflammation rapidly subsided. Histologic examination of the two tumors on the ears disclosed

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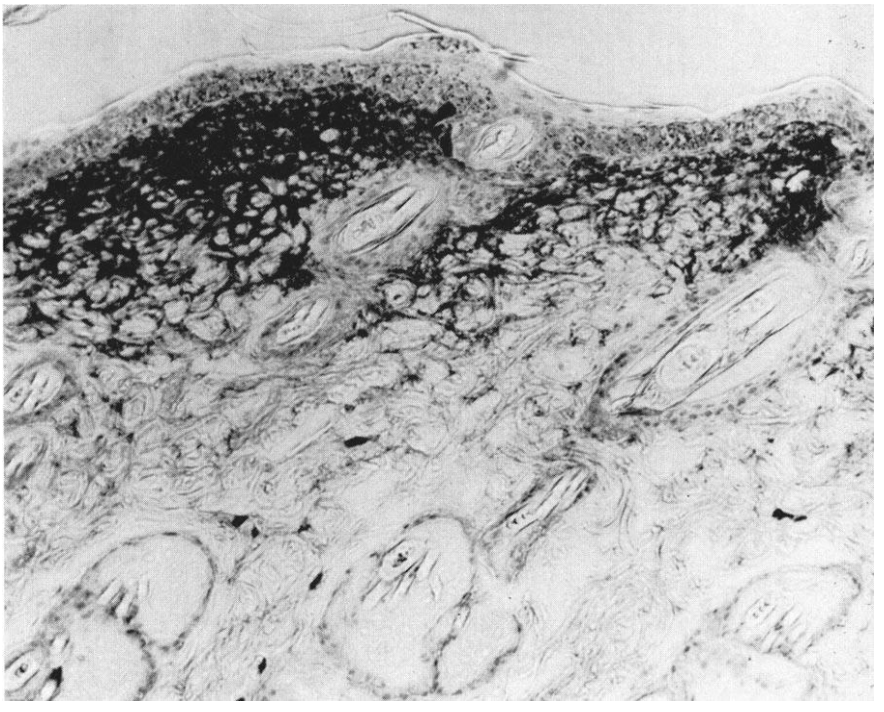

FIG. 1. Prominent increase in ground substance acid mucosaccharide is present after 7 weeks of UVL. Colloidal iron, × 120.

simple papillomas. No abnormal changes were seen in the control animals.

Hematoxylin and eosin, and PAS-stained sections were used to study collagen fibers. Snook's method was used for evaluation of reticular fibers. An increased number of reticular fibers appeared in the superficial corium after UVL was discontinued. Mild fibrosis of collagen fibers was observed in the superficial corium in most sections taken at 46 weeks.

Acid mucosaccharides of the extracellular interfibrillar ground substance began showing a prominent increase in the superficial corium after 3 weeks of exposure to UVL. The amount of acid mucosaccharide increased for several weeks and then remained relatively constant during the remainder of the exposure period (Figs. 1 and 2). The quantity of the ground substance acid mucosaccharide gradually decreased toward normal after UVL was stopped. The histochemical properties of the acid mucosaccharide were as follows: colloidal iron positive and partially hyaluronidase labile, alcian blue positive at pH 2.5 and negative at 0.4, and aldehyde-fuchsin negative. Initially, most of the ground substance acid mucosaccharide was removed by hyaluronidase digestion; but after several weeks a larger proportion of this material was hyaluronidase resistant. The above reactions indicate the presence of hyaluronic acid and possible other unidentifiable mucosaccharides.

Dilatation and increased tortuosity of the blood vessels in the superficial corium were observed in alkaline phosphatase preparations of animals exposed to UVL after 7 weeks. After 15 to 17 weeks of UVL exposure, alkaline phosphatase positive fibrocytic cells were seen about the papillary capillaries, and subsequently, diffuse alkaline phosphatase activity appeared in the stroma about the capillaries. The blood vessels of control animals were normal and fibrocytes were alkaline phosphatase negative.

Elastic tissue was evaluated with aldehyde-fuchsin stained cryostat sections, and with aldehyde-fuchsin and the Movat stained paraffin tissue sections. The aldehyde-fuchsin stained cryostat sections gave the best demonstration of elastic tissue after UVL exposures. Elastic tissue in the superficial corium gradually diminished as the inflammatory cellular infiltrate

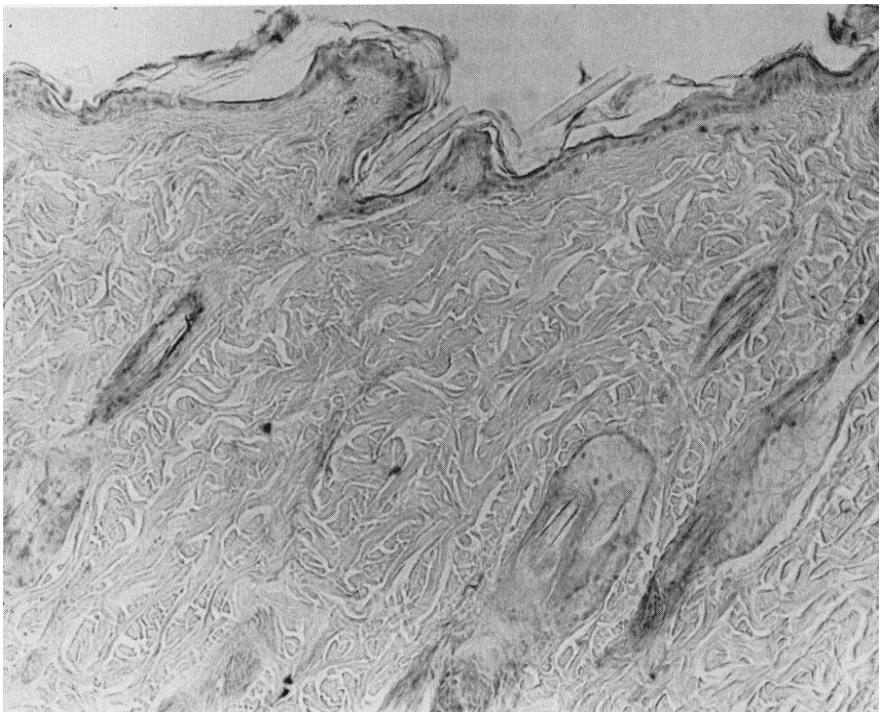

FIG. 2. Normal skin of control animal at 7 weeks for comparison with Fig. 1. Colloidal iron, × 120.

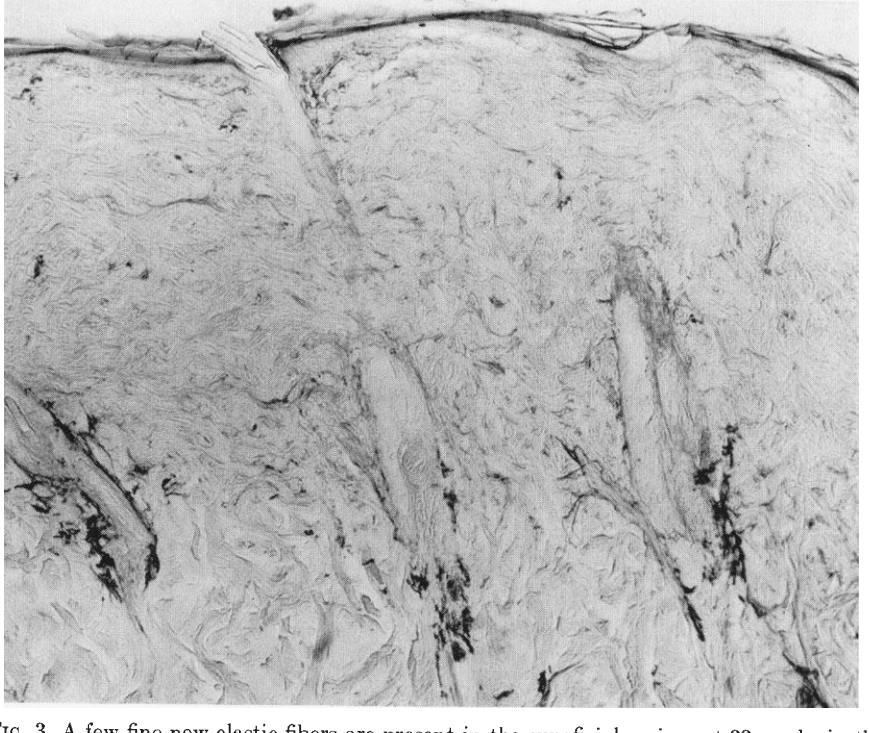

FIG. 3. A few fine new elastic fibers are present in the supeficial corium at 33 weeks in the area where the elastica was previously destroyed by inflammation: Elastic fibers adjacent to the hair follicles seen in the middle corium generally remained intact and were not destroyed by the infiltrate. Aldehyde-fuchsin pH 1.7, × 92.

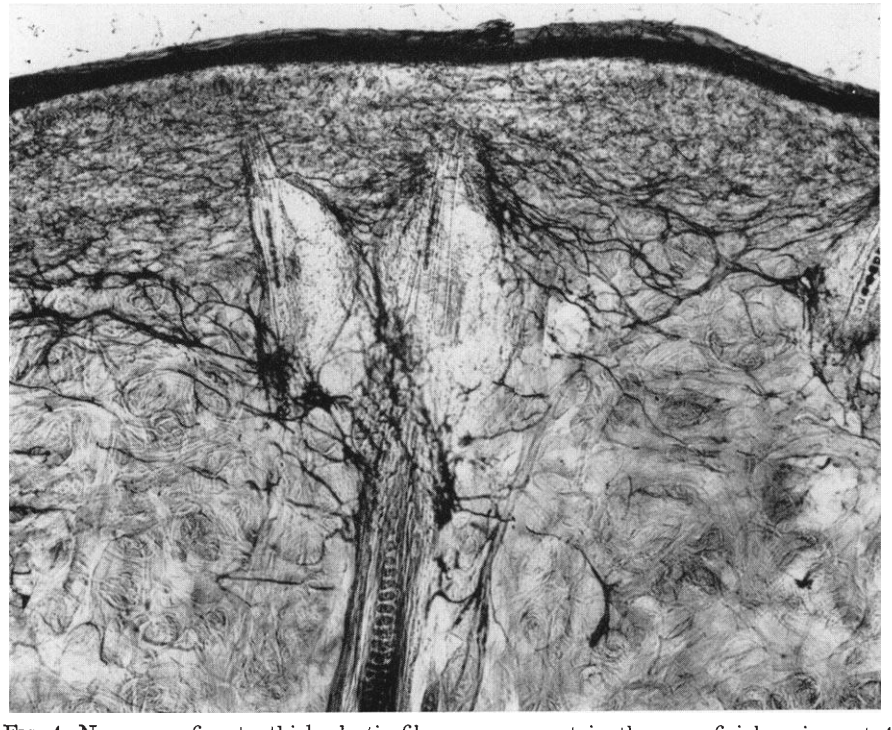

FIG. 4. Numerous fine to thick elastic fibers are present in the superficial corium at 46 weeks. Aldehyde-fuchsin pH 1.7, × 92.

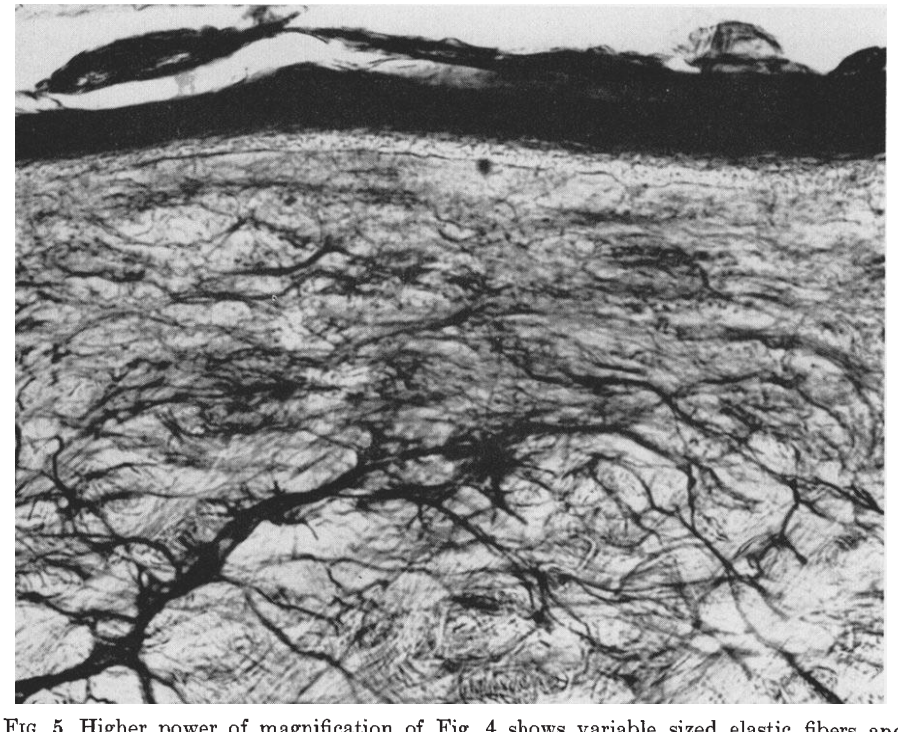

FIG. 5. Higher power of magnification of Fig. 4 shows variable sized elastic fibers and some interfibrillar reactive amorphous material. Aldehyde-fuchsin pH 1.7, × 165.

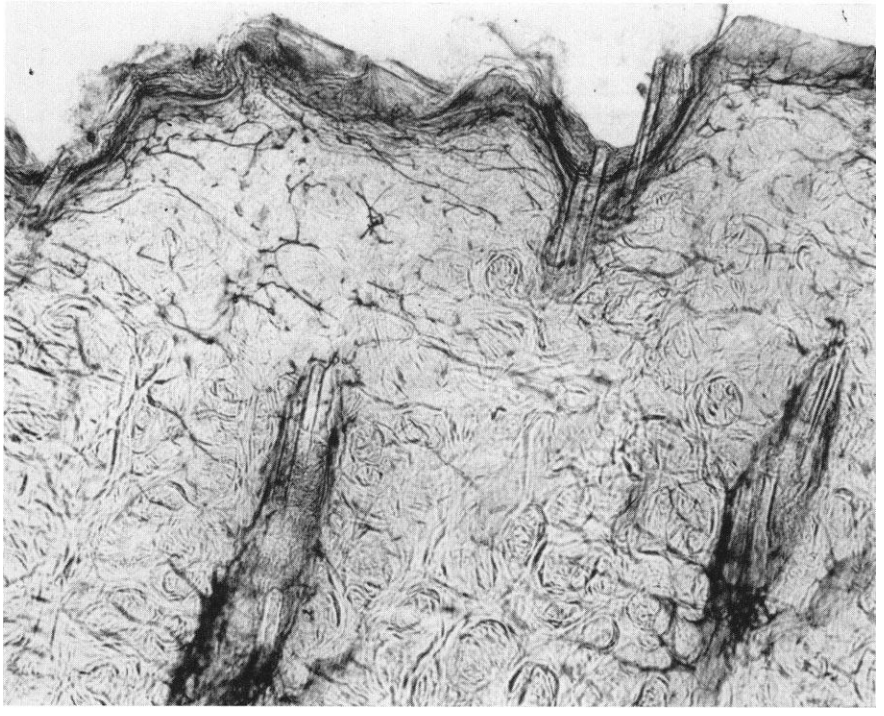

FIG. 6. Normal skin of control rat at 46 weeks for comparison with the elastic fiber changes demonstrated in Figs. 3, 4 and 5. Aldehyde-fuchsin pH 1.7, × 92.

increased. Most of the elastic fibers in the superficial corium were destroyed by 23 weeks and remained virtually absent until the UVL was discontinued. Specimens obtained at 33 weeks (Fig. 3) showed formation of new elastic tissue in the superficial corium as fine fibers and these increased in number and thickness throughout the remainder of the period of observation to 46 weeks. Dense collections of elastic fibers were located in the superficial corium and about the hair follicles and these changes were separated from the epidermis by a narrow grenz zone. The amount of elastic tissue in the superficial corium at 46 weeks (Figs. 4 and 5) was significantly greater than that seen in the control untreated animals (Fig. 6). Some tissue sections obtained at 46 weeks from animals exposed to UVL showed granular or amorphous interfibrillar material which was reactive with elastic stains and was localized to the areas of elastotic change. The grenz zone, the elastotic changes, and ground substance alterations closely resembled solar elastosis of human skin.

COMMENT

Dilatation and tortuosity of capillaries in the superficial corium appeared relatively early after exposure to UVL and these vascular changes probably represent a direct response to irradiation. We have observed similar changes in papillary capillaries seen in solar elastosis and precancerous dermatoses of human skin.

An increase in ground substance acid mucosaccharide with histochemical properties of hyaluronic acid was a striking early change. Mathieson and Pearce (7) performed chemical analysis of the acid mucosaccharide of the ground substance of normal rat skin. They found hyaluronic acid to be present but were unable to detect chondroitin sulfates. The amount of acid mucosaccharide decreased prior to and during the period of increased elastic tissue formation. Sams, Smith and Burk (1) also observed an increased amount of acid mucosaccharide in the superficial corium in mice treated with ultraviolet light. Previous studies (6) indicate dermal fibrocytes represent one source of hyaluronic acid and that an increased

production of this acid mucosaccharide is usually associated with a decreased production of collagen and elastica. Not all of the colloidal iron positive material which accumulated in the superficial corium in rats exposed to UVL was removed by hyaluronidases digestion; this is similar to our experience that not all of the colloidal iron positive material associated with solar elastosis of humans is removed by short periods of digestion with hyaluronidase (8).

The delay of elastic tissue proliferation was probably due to inflammation present during the period of UVL exposure since the cellular infiltrate destroyed much of the normal elastic fibers present and apparently retarded new fiber formation. The results obtained in our study stress the necessity of stopping exposure to UVL before a significant increase in elastic tissue production occurs in rat skin, and probably explain why most attempts at experimental production of dermal elastosis in animals have been unsuccessful. The grenz zone observed in our study was also reported by Sams, Smith and Burk (1) and this change is commonly seen in human solar elastosis.

Our histopathologic and histochemical studies show evidence of increased elastic tissue production in the superficial corium in rats exposed to UVL and these changes are similar to solar elastosis in man after chronic actinic irradiation. The question as to whether other types of injury may also produce elastosis is unsettled. An increase in elastic fibers has been reported as occurring following application of carcinogens to mouse skin (9). Sams, Smith and Burk (1) did not find evidence of elastosis in mice treated with a primary irritant. Our results indicate a need for additional evaluation of experimental production of elastosis. We plan to further evaluate this problem by use of primary irritant and carcinogens as controls; chemical analysis of tissue showing histochemical evidence of elastosis; and varying the amount and intervals of exposure to ultraviolet light.

SUMMARY

The effect of UVL on connective tissue of rat skin was studied using histopathologic and histochemical methods. Thirty female albino rats were exposed to UVL 3 days a week for 27 weeks. Biopsies were taken from UVL exposed and control animals every two weeks during a 46 week period. A prominent increase in acid mucosaccharide with histochemical properties of hyaluronic acid occurred in the superficial corium after three weeks of UVL exposure and diminished when the UVL was stopped. Alkaline phosphatase studies demonstrated significant vascular changes, reactive dermal fibrocytes, and focal diffuse alkaline phosphatase activity in the stroma of the papillary corium. As edema and cellular infiltration in the superficial corium increased elastic fibers disappeared and remained virtually absent while exposure to light was continued. New elastic tissue fibers appeared in the superficial corium after UVL was stopped and elastica gradually increased to abnormal quantities closely simulating solar elastosis of human skin.

REFERENCES

1. Sams, W. M., Jr., Smith, J. G. and Burk, P. G.: The experimental production of elastosis with ultraviolet light. J. Invest. Derm., *43:* 467, 1964.
2. Menne, H. L. and Johnson, W. C.: A resinous mounting medium compatible with azo-dye staining for alkaline phosphatase. Stain Technol., *41:* 295, 1966.
3. Johnson, W. C.: Histochemistry of the cutaneous ground substance, pp. 35–51, *Methods and Achievements in Experimental Pathology,* Vol. 1. Eds., Bajusz, E. and Jasmin, G. G., Karger, Basel and New York, 1966.
4. Movat, H. Z.: Demonstration of all connective tissue elements in a single section. Arch. Path. (Chicago), *60:* 289, 1955.
5. Armed Forces Institute of Pathology. *Manual of Histologic and Special Staining Technics,* 2nd Ed. The Blakiston Division, McGraw-Hill Book Company, Inc., New York, 1960.
6. Johnson, W. C. and Helwig, E. B.: Cutaneous focal mucinosis. Arch. Derm., *93:* 13, 1966.
7. Mathieson, J. M. and Pearce, R. H.: The glycosaminoglycan of the ground substance of rat skin. Canad. J. Biochem., *41:* 2327, 1963.
8. Johnson, W. C. and Helwig, E. B.: Histochemistry of the acid mucopolysaccharides of skin in normal and certain pathologic conditions. Amer. J. Clin. Path., *40:* 123, 1963.
9. Ma, C. K.: Morphological and chemical investigation of dermal elastic, and collagenic tissue during epidermal carcinogenesis. Cancer Res., *9:* 481, 1949.