ticosterone acetate into fasted adrenalectomized rats does prevent the drop in concentration of blood sugar found in untreated adrenalectomized rats. The amount required to maintain the blood sugar is greater than that necessary to maintain normal concentrations of serum electrolytes and non-protein nitrogen.

10957

Effect of Local Applications on Development of Ultraviolet Tumors.*

HAROLD P. RUSCH, CARL A. BAUMANN AND BERNERD E. KLINE.
(Introduced by W. J. Meek.)

From the Department of Physiology, University of Wisconsin Medical School, Madison.

We have previously demonstrated that the rate of tumor production by ultraviolet light varies with the time of irradiation, with the amount of pigment in the skin, and with the character of the diet.[1, 2] The present study deals with the effect of various substances on the development of U. V. tumors, when applied directly to tissues in which neoplastic changes are occurring.

Three types of materials were used: oils, oxidizing agents, and carcinogenic compounds. Oils were studied because previous work had shown that the production of U. V. tumors was markedly accelerated by the consumption of a high-fat diet (30% Crisco), on which the fur became definitely greasy. The acceleration, therefore, could have been due to either a local or a systemic effect. By painting oil on the ears of irradiated mice on a normal diet the local factor alone could be studied.

The oxidizing agents were studied because of Roffo's emphasis of the importance of cholesterol in tumor production. He has shown that irradiated cholesterol fogs a photographic plate[3] and claims that this reaction is analogous to that taking place within the skin, when an animal is exposed to excess amounts of ultraviolet light. Stavely and Bergmann[4] and Mayneord and Roe[5] have demonstrated

* Supported in part by the Jonathan Bowman Cancer Fund and the Wisconsin Alumni Research Foundation.

1 Rusch, H. P., and Baumann, C. A., *Am. J. Ca.*, 1939, **35**, 55.

2 Baumann, C. A., and Rusch, H. P., *Am. J. Ca.*, 1939, **35**, 213

3 Roffo, A. H., *Am. J. Ca.*, 1933, **17**, 42.

4 Stavely, H. E., and Bergmann, W., *Am. J. Ca.*, 1937, **30**, 749.

5 Mayneord, W. V., and Roe, E. M. F., *Am. J. Ca.*, 1937, **31**, 476.

that irradiated cholesterol contains peroxides which are slowly liberated, and that the fogging of a photographic plate by irradiated cholesterol is due to these liberated peroxides. We have, therefore, painted known peroxides, as well as cholesterol solutions, on the ears of mice developing U. V. tumors.

Carcinogenic compounds were studied as a means of obtaining information on the interrelation of cancer-producing agents such as chemicals and light. This interrelationship is as yet poorly understood, and available data are both incomplete and contradictory. Schorr and Ssobolewa[6] and Vles, DeCoulon, and Ugo[7] report a more rapid development of tumors due to tar painting in mice kept in the light as compared to those kept in the dark. Bang,[8] Lipschütz,[9] and Seelig and Cooper,[10] however, failed to find any such difference. Findlay[11] found that cancer development in tarred mice was accelerated by ultraviolet light, and Büngeler reported a similar effect due to sunlight.[12] However, Kohn-Speyer[13] failed to confirm Findlay; and Taussig, Cooper, and Seelig[14] observed that ultraviolet light did not stimulate tumor production in mice painted with benzpyrene. The discrepancies are no doubt partly due to differences in the relative amounts of light and carcinogenic agents employed. In our present study the amount of light used was by itself sufficient to produce tumors in from 4 to 6 months.

Tumors were produced in mice by irradiation with ultraviolet light, and during the induction period, various substances were painted on the ears, *viz.*, those tissues in which the tumors were developing. The technic of irradiation was essentially that described previously.[1] Young adult strain C mice† of both sexes were used throughout. They were placed in groups of 20 to 25 and were irradiated in cylindrical wire mesh cages for 45 minutes daily, at a distance of 50 cm from an air-cooled mercury vapor lamp. The experiments were run in series over a period of 16 months. Each series

6 Schorr, G., and Ssobolewa, N., *Z. f. Kreb.*, 1930, **31**, 308.

7 Vles, F., DeCoulon, A., and Ugo, A., *Compt. rend. Acad. d. Sc.*, 1931, **193**, 893.

8 Bang, F., *Compt. rend. Soc. de Biol.*, 1922, **87**, 754.

9 Lipschütz, B., *Arch. Dermat. u. Syph.*, 1924, **147**, 161.

10 Seelig, M. G., and Cooper, Z. K., *Surg., Gyn. and Ob.*, 1933, **56**, 752.

11 Findlay, G. M., *Lancet*, 1928, **2**, 1070.

12 Büngeler, W., *Z. f. Krebs.*, 1937, **46**, 130.

13 Kohn-Speyer, A. C., *Lancet*, 1929, **2**, 1305.

14 Taussig, J., Cooper, Z. K., and Seelig, M. G., *Surg., Gyn. and Ob.*, 1938, **66**, 989.

† Obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

TABLE 1.
Effect of Oils, Carcinogens and of Peroxides on Tumor Production.

Substance	Conc. and solvent	No. of mice at start	No. of mice at time of first tumor	Animals rayed	Chemicals rayed	% of animals with tumors									
						3 Mo.	3½ Mo.	4 Mo.	4½ Mo.	5 Mo.	5½ Mo.	6 Mo.	6½ Mo.	7 Mo.	7½ Mo.
Controls	—	65	57	+	—	0	6	17	29	52	63	70	90	—	—
Mineral oil	100%	40	40	+	0	5	22	49	89	98	—	—	—	—	—
,, ,,	100%	25	21	0	+	0	0	0	0	0	0	0	0	0	0
Cholesterol	3% in cottonseed oil	20	19	+	0	0	28	44	72	100	—	—	—	—	—
Cottonseed oil	100%	40	38	+	0	0	5	22	56	77	85	—	—	—	—
Olive oil	100%	20	18	+	0	0	6	28	56	83	—	—	—	—	—
Wheat germ oil	Crude ether extr.	20	19	+	0	0	11	17	50	83	—	—	—	—	—
Linseed oil	100%	20	20	+	0	0	5	10	20	40	—	—	—	—	—
Methylcholanthrene	0.2% in benzene	20	20	0	0	0	0	10	30	50	60	70	100	—	—
,,	0.2% in cottonseed oil	20	17	0	0	0	6	12	18	47	56	65	71	—	—
,,	0.2% in mineral oil	20	19	0	0	0	0	0	0	33	48	55	78	—	—
1,2,5,6 Dibenzanthracene	0.3% in benzene	21	21	+	0	0	5	15	39	50	66	66	83	—	—
,, ,,	,, ,, ,,	21	19	0	0	0	0	0	0	0	0	0	12	—	—
,, ,,	,, ,, ,,	21	20	0	+	0	0	0	0	0	0	0	0	0	0
Controls*	—	25	24	+	0	0	4	8	10	10	30	45	50	60	70
Cholesterol*	3% in benzene	25	24	+	0	0	0	0	5	16	21	53	63	74	80
,, *	,, ,, ,,	20	19	0	+	0	0	0	0	0	0	0	0	0	0
Benzene*	100%	25	24	+	0	0	0	0	0	6	30	45	50	50	55
Benzoyl peroxide*	1% in benzene	25	23	+	0	0	0	0	5	10	15	25	45	50	70
,, ,, *	,, ,, ,,	20	18	0	0	0	0	0	0	0	0	0	0	0	0
Hydrogen peroxide*	6% in 50% glycerol	25	20	+	0	0	0	0	6	6	17	33	42	47	58
,, ,, *	,, ,, ,, ,,	20	20	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol*	100%	25	23	+	0	0	0	0	5	5	10	30	30	35	35

* To be compared with the second control series.

contained a group of non-treated irradiated controls, and the results of each group were then compared with its own control.

The animals were painted 3 times weekly, a camel's hair brush being used for the applications. The oils painted were wheat germ oil, cottonseed oil (Wesson oil), olive oil, linseed oil, and mineral oil. The wheat germ oil was a crude ether extract of wheat germ prepared in our laboratory. The other oils were purified oils as sold commercially, the mineral oil being of the grade used clinically. The peroxides applied were benzoyl peroxide‡ and hydrogen peroxide (Superoxal diluted with 50% glycerol to a 6% solution). The cholesterol‡ was recrystallized from alcohol. The carcinogenic chemicals were methylcholanthrene‡ and 1, 2, 5, 6 dibenzanthracene.‡ The concentrations of the various substances and the solvents used are given in Table I.

To determine whether the accelerating carcinogenic effect of the applied substances was a synergistic one, or due to the production of carcinogenic agents in the irradiated material, various oils and solutions were irradiated prior to application, and then painted onto the ears of non-irradiated animals. The solutions were irradiated in flat-bottom containers for one hour every week at a distance of 50 cm from an air-cooled Hg-vapor lamp. Solutions were prepared fresh every week. The animals were maintained on a diet of Purina dog chow and water *ad libitum,* and were examined every 2 weeks for neoplastic changes. Only definitely malignant growths were considered "tumors"; early thickenings and warts were classified as "intermediate". Results are expressed as the percentage of tumors at any given time—that is:

$$\frac{\text{(number with tumors)}}{\text{(number alive when first tumor develops)}} \times 100.$$

Most of the oils applied accelerated the rate of tumor production with ultraviolet light. Mineral oil was the most active, although cottonseed oil, olive oil, and wheat germ oil also had some effect. For example, after 4½ months of irradiation, tumors were present in 29% of the control animals, in 89% of those receiving mineral oil, in 56% of those receiving cottonseed oil or olive oil, and in 50% of those receiving wheat germ oil. Linseed oil retarded the production of ultraviolet tumors, probably due to the formation of a film of oxidized oil which decreased the penetration of the light. Glycerol also retarded tumor production somewhat. The stimulating action of oils on tumor production, therefore, did not appear to be due to a basic chemical property of the glyceride molecule, but rather

‡ Eastman Kodak Company.

to the physical characteristics of the oil—since mineral oil was the most active oil found. Mineral oil, however, did not appear to possess general carcinogenic properties. Irradiated mineral oil applied to non-irradiated animals failed to produce tumors and furthermore mineral oil failed to stimulate the production of tumors with methylcholanthrene. Methylcholanthrene was equally effective when painted in benzene or in cottonseed oil, but tumor production was actually retarded when it was applied in mineral oil. The stimulating action of mineral oil on tumor development, therefore, appears to be confined to ultraviolet tumors. The mechanism of this stimulation is unknown, but it is possibly due to a softening of the skin, permitting the penetration of more light to the active basal layer. It is also possible that the activity of the cells themselves is altered by the presence of oil.

Cholesterol markedly stimulated tumor development when applied in cottonseed oil but not when applied in benzene. Others have noted the formation of peroxides when cholesterol is irradiated in air[4, 5] and we have made a similar observation. However, the stimulating action of cholesterol on tumor development is probably not due to peroxides, since neither hydrogen peroxide, benzoyl peroxide, nor irradiated cholesterol were carcinogenic when applied to the ears of non-irradiated animals, nor did the presence of known peroxides accelerate the development of ultraviolet tumors. Furthermore, mineral oil, which accelerates the formation of ultraviolet tumors, does not develop peroxides on irradiation. It is possible that the extra amounts of cholesterol in the cottonseed oil enhanced the penetration of the oil into the skin, thus in effect increasing the action of the oil.

Our results with dibenzanthracene appear to be in line with those of Taussig, Cooper, and Seelig,[14] who found that ultraviolet light did not increase the carcinogenic activity of benzpyrene. However, they used relatively little light. It is of interest in this connection to note that dibenzanthracene, methyl cholanthrene, and benzpyrene all showed photodynamic activity when tested on *coleps*[15] indicating that all 3 are capable of producing biological changes in conjunction with light.

Summary. The rate of tumor production with ultraviolet light could be altered by the local application of certain substances to the tissues developing tumors. Of the oils tried, mineral oil accelerated tumor development most rapidly; cottonseed oil, olive oil, and wheat germ oil caused slight acceleration; linseed oil retarded tumor formation. Cholesterol in oil caused marked acceleration. Peroxides were without effect. 1,2,5,6, dibenzanthracene was also without effect.

[15] Mottram, J. C., and Doniach, I., *Lancet*, 1938, **1**, 1156.