The Water Content in the Epidermis of Mice Undergoing Carcinogenesis by Methylcholanthrene*

V. Suntzeff, M.D., and Christopher Carruthers, Ph.D.

(From The Barnard Free Skin and Cancer Hospital, St. Louis 3, Missouri, and the Department of Anatomy of Washington University School of Medicine, St. Louis 10, Missouri)

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Studies on the role of the minerals in epidermal carcinogenesis of mice induced by methylcholanthrene have been described (2), and the need for the determination of the water content of the epidermis became obvious in investigations on the succinic dehydrogenase and cytochrome oxidase activities of the epidermis (3). Since the latter are expressed as cu. mm. of oxygen consumed per mgm. dry weight of tissue per hour, it became imperative to ascertain in what way the water content behaved during carcinogenesis so that the activities of the enzymes could be placed on a more adequate basis of reference.

In our previous studies the epidermis was separated from the dermis at 50° C., and no attempt was made to determine the water content since one would expect some loss of water at this temperature. We have overcome this obstacle through separating epidermis from the dermis at room temperature by the following procedure: A block of wood was covered with a sheet of plastic and the whole mouse skin, fur side up, was stretched tightly over it with pins. With a small dull scalpel the epidermis was scraped off the dermis. Histological examination of the dermis revealed that it was not disturbed, although a few epidermal cells were left in contact with it. Fig. 1 shows the procedure for the separation process.

The water content of normal, untreated epidermis was determined by drying the sample to constant weight at 105° C., and also by the Bidwell-Sterling toluene extraction method. With the former procedure the epidermis upon removal from the dermis was placed into a small, round-bottom, stoppered-flask, which was submerged in a mixture of ice and water to minimize loss of water by evaporation. The sample was then weighed and dried to constant weight at 105° C. A micro Bidwell-Sterling apparatus was constructed with Pyrex glass and the receiving vessel was calibrated with mercury. The samples of epidermis were removed as described above and then, after weighing, were refluxed with dry toluene until no more water fell into the receiving tube. Since the water content of normal epidermis was 60.9 per cent with the former method as compared with 59.8 per cent with the latter and therefore practically the same by both procedures (Table I), all samples of hyperplastic epidermis and tumors were dried at 105° C. to determine their water content.

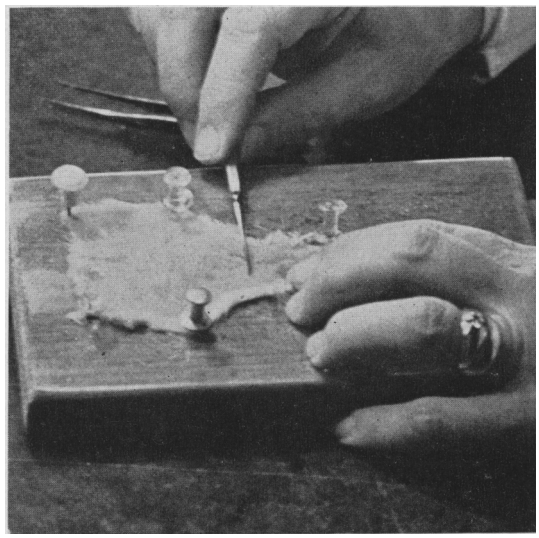

FIG. 1.—Skin stretched on block for removal of epidermis.

The mice were shaved and treated with the carcinogen as previously described (2), and all animals were sacrificed 5 days after the last application of the carcinogen. For the final stages of carcinogenesis the transplantable squamous cell carcinoma of Cooper, Firminger, and Reller was used (4).

RESULTS

The results are shown in Table I and are expressed as the percentage of water in the different samples. The water content of the normal untreated epidermis

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was 60.9 per cent by drying at 105° C., and 59.8 per cent by the toluene extraction method. Two of the samples in the former group (marked with an asterisk)

TABLE I: WATER CONTENT IN EPIDERMAL METHYLCHOLANTHRENE CARCINOGENESIS

Number of mice	Number of paintings	Time after first treatment to killing of mice, days	Water content, %
NORMAL UNTREATED MICE			
DETERMINATION BY DRY WEIGHT			
15			60.0
15			60.4
18			62.6
*12			61.9
*12			59.5
72 (total)			Average 60.9
DETERMINATION BY EXTRACTION WITH TOLUENE			
15			62.8
15			56.0
15			58.5
15			62.0
60 (total)			Average 59.8
METHYLCHOLANTHRENE-TREATED MICE			
9	3	10	64.4
11	3	10	66.5
8	3	10	68.6
28 (total)			Average 66.5
9	6	17	64.0
8	6	17	67.2
9	6	17	68.7
8	6	17	63.0
6	6	17	65.2
40 (total)			Average 65.6
8	12	31	68.0
10	12	31	67.3
10	12	31	66.3
28 (total)			Average 67.2
6	24	62	66.2
4	24	62	64.0
6	24	62	67.5
16 (total)			Average 65.9
TUMORS			
5			81.7
6			82.5
5			80.5
16 (total)			Average 81.6

* These samples of epidermis were removed at 50° C.

were removed at 50 to 51° C., and gave about the same water content as those removed at room temperature, showing that little if any loss of water occurs at the higher temperature. Mice that received 3 and 6 applications of methylcholanthrene contained 66.5 and 65.6 per cent water respectively, and those that were painted 12 and 24 times had 67.2 and 66.7 per cent water respectively. The hyperplastic epidermises contained more water than did the normal. Three samples of squamous cell carcinoma had an average of 81.7 per cent water, a considerable increase over the normal and hyperplastic epidermises. That tumors contain more water than the tissues from which they develop is well known (7).

The variation in the water content with time and the number of applications of the carcinogen is shown graphically in Fig. 2, which demonstrates that the water content of hyperplastic epidermis is not only higher than normal, but remains higher and constant through the precancerous stages. A much greater increase in the water content occurs when the epidermal cells become carcinomatous.

Microscopic studies by others in the Barnard Hospital group provide data that seem to be correlated with these findings. Thus, the nucleocytoplasmic ratio of basal and spinous cells decreases promptly in the early stages of epidermal hyperplasia and remains at approximately the same figure for 50 days (5). This is mainly due to an increase in cytoplasm which is occasioned perhaps, to some extent, by an increase in water. In addition, the definite increase in the displaceability of nuclear chromatin and nucleoli of epidermises subjected to ultracentrifugal force during early hyperplastic stages in the response to the carcinogen and the greater and more uniform increase in displaceability in the resulting cancer (6) may well be associated in some measure with the increase in water content.

DISCUSSION

The greater amount of water in tumors is usually attributed to the following factors (7): (a) The presence of hydration-promoting ions, potassium, sodium, chloride; (b) the presence of similarly effective protein decomposition products; (c) a change of the lipid composition, which influences cell permeability.

The relation, cholesterol/lipid phosphorus, is believed by Meyer and Schaeffer (1) to be a measure of the ability of a tissue to hold water; that is, the higher the value of the ratio the greater the water content of the tissue. Our integrated program of studies on epidermal carcinogenesis showed no appreciable change in the sodium and potassium contents in hyperplastic

epidermis, and slight decrease in the content of these metals in the carcinoma derived from the epidermis (2). Yet in this paper we report a slight increase in the water content in the hyperplastic epidermis and a signal increase in the carcinoma. With respect to the cholesterol/lipid phosphorus ratio, Wicks and Suntzeff (8) have demonstrated a decrease in the cholesterol protein nitrogen ratio in hyperplastic mouse epidermis, and recent studies in this laboratory have shown that the phospholipid content likewise drops in methylcholanthrene-treated epidermis,[1] and yet the water content is somewhat increased. Since the tumors that we employ for analysis are small, solid, and show little if any necrosis microscopically, it would appear that the presence of protein decomposition products would not adequately account for the increased water content in the carcinoma.

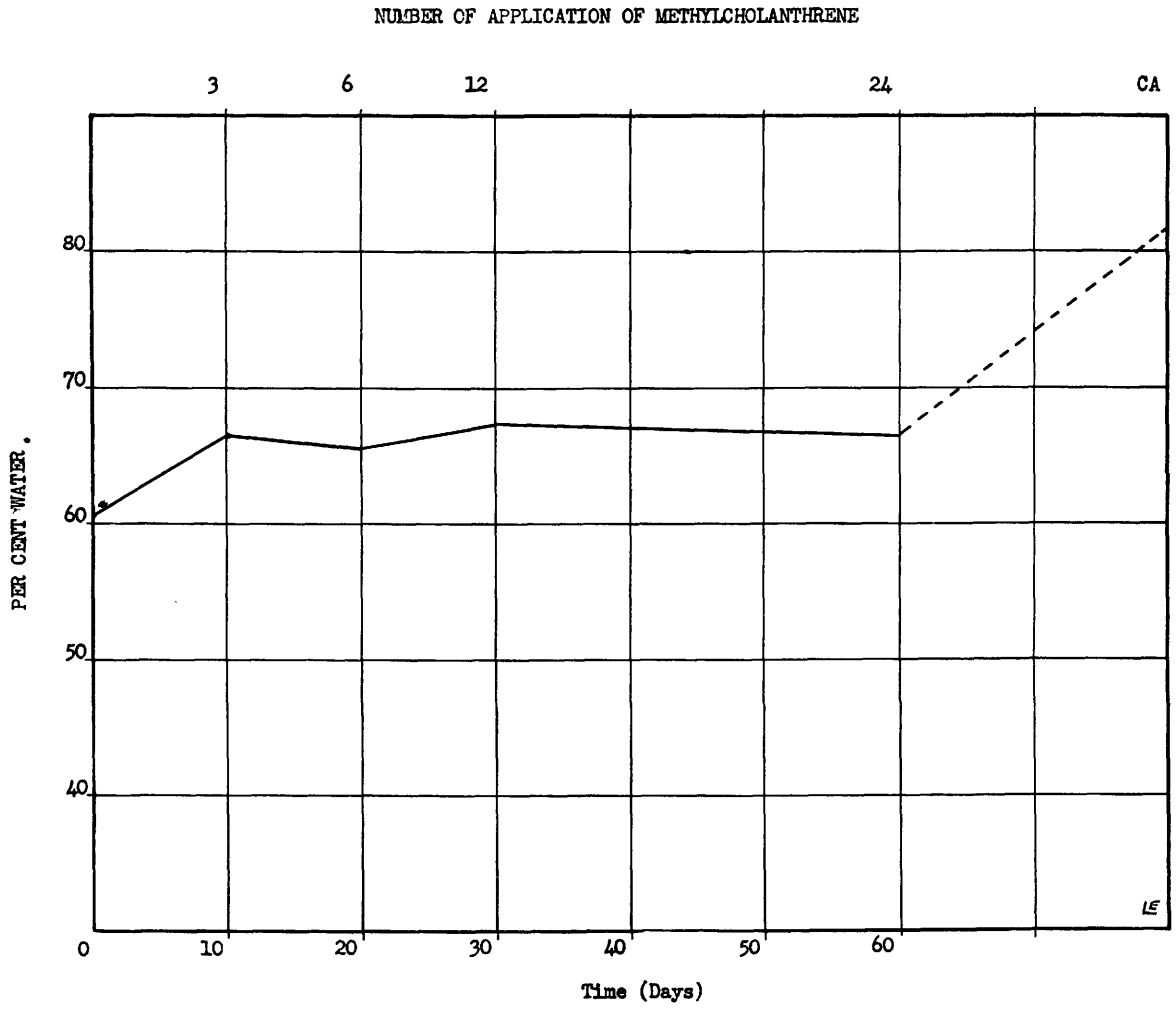

FIG. 2.—The water content in epidermal methylcholanthrene carcinogenesis.

[1] Unpublished observations—R. L. Simoes, C. Carruthers, C. J. Costello, and M. Kamen. "Uptake of P_{32} in the Phospholipid Fraction of Mouse Epidermis Undergoing Carcinogenesis by Methylcholanthrene."

SUMMARY

The water content of normal untreated mouse epidermis, methylcholanthrene-treated epidermis, and of a squamous cell carcinoma, originally derived from mouse skin, has been determined. The water content of normal mouse epidermis was 60.3 per cent, while the average of hyperplastic epidermises, which were practically the same throughout this stage was 66.5 per cent. The carcinoma contained an average of 81.7 per cent water.

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