ing infusion of fat in diabetic animals deprived of insulin. Serum cholesterol concentration falls following infusion of fat into pancreatectomized animals receiving insulin. Observations on fat metabolism in which the fat is administered intravenously give data comparable to those obtained following oral administration of fat and the mechanisms of clearance seem identical by the two methods.

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Amperometric Determination of Sulfhydryl Changes in Mouse Epidermis and Dermis During Treatment with Certain Polycyclic Hydrocarbons.* (23447)

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Investigation of sulphur metabolism in biological systems is challenging because of its integration with cell activities. Currently, the relationship between sulfhydryl groups and the carcinogenic process is receiving much attention. Polycyclic carcinogenic hydrocarbons have been presumed to interfere with some phase of sulphur metabolism related to growth. Reimann and Hall(1) showed that parathiocresol inhibited induction of tumors by 1,2,5,6-dibenzanthracene. They concluded that the sulfhydryl group of parathiocresol had an anti-carcinogenic effect. Fieser(2) discovered a close relationship between carcinogenicity and certain types of substitution reactions at positions in the molecule. He suggested a reaction of the hydrocarbon *in vivo,* whereby an S-S linkage of a protein or peptide is broken, giving rise to a free sulfhydryl group and a hydrocarbon-peptide link. Later, Crabtree(3,4) suggested that during carcinogenesis, inhibition may occur by some interference with sulphur metabolism. Crabtree postulated that in the production of a skin tumor by a carcinogen, the initial step involved was the fixation of the carcinogen by combination with free sulfhydryl groups.

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Others(5) have demonstrated that the carcinogenic hydrocarbons react with RSH groups. DiPaolo, Hill and Stanger(6) reported that some carcinogenic and non-carcinogenic polycyclic hydrocarbons reduced the amount of soluble SH in treated skin of mice.

The purpose of the present investigation was to determine amperometrically the changes in the total sulfhydryl content, in protein sulfhydryl content, and by subtraction, in the soluble sulfhydryl content of mouse epidermis and dermis which were treated with certain carcinogens or with anthracene.

Materials and methods. The polycyclic carcinogenic hydrocarbons employed in this work were 1,2,5,6-dibenzanthracene (DBA), 9,10-dimethyl-1,2-benzanthracene (DMBA) and anthracene. The concentrations of DBA and DMBA were 0.05% (weight-volume%) in acetone solution and the concentration of anthracene was 0.10%. The mixture of two carcinogens consisted of 0.05% DMBA and 0.10% DBA in the same solvent.

Female Bagg albino mice 3½ months old were obtained from our own colony. Immediately before application of acetone solutions, hair was removed from the dorsal skin with electric clippers and the mice were selected in which the dorsal hair appeared to be in the telogen (resting) phase of the hair cycle. To secure an adequate sample a pair of mice was used for each determination. Two-hundredths ml of solution was applied to the clipped skin of each test mouse with a calibrated pipette. (0.02 ml of a 0.05% solution equal 10 μg of solute.) At selected intervals, pairs of mice were killed by cervical dislocation and the treated skin was promptly removed. The subcutaneous fat and connective tissue were scraped off with a scalpel. To separate dermis from epidermis, the scraped whole skin was placed on a piece of clean polyethylene sheeting. After the skin was stretched, the epidermis was scraped off with a scalpel(7). The epidermis and the dermis from each pair were minced separately with scissors, placed in a small beaker, and weighed. The mince was transferred to a Potter homogenizer immersed in ice water. The weight of the sample was determined by difference.

After being homogenized with 10 ml of glass-distilled water, the homogenate was transferred to a plastic centrifuge tube, and the washings from an additional 5 ml of water were added. The suspension was centrifuged in a refrigerated International Centrifuge, Model PR-2 with a high speed-head for 15 minutes at 18,000 rpms, at a temperature of 5°C. The supernatant solution was filtered with No. 41 Whatman filter paper. The total sulfhydryl content and the protein sulfhydryl content were determined by titration with 0.001 M $HgCl_2$ solution, according to the technic of Kolthoff, Stricks, and Morren(8). The primary reaction in the mercurimetric amperometric titration is the binding of one mercury atom by one SH group.

This procedure utilized a rotating platinum electrode (connected with a Sargent Ampot) inside a 50 ml beaker containing 15 ml of phosphate buffer(8). Pure nitrogen was bubbled through this solution until the residual current (with an applied EMF of -0.2V *vs.* saturated calomel electrode) was less than 0.5 μamp. For total SH determination, a 5 ml aliquot of the water filtrate was added to 15 ml of phosphate buffer low in oxygen in a 50 ml beaker and titrated as above.

For the protein SH determination, 1 ml of 10 M trichloroacetic acid was added to a second 5 ml aliquot of water filtrate. This sample was centrifuged as before, the supernate discarded and the remaining protein precipitate dissolved by adding 1 ml of 1 N NaOH. Following neutralization of the sample with 2 N HCl, the solution was titrated amperometrically using phosphate buffer as previously described. The results were calculated as mg of SH per 100 mg net weight of either dermis or epidermis (one ml of 10^{-3} M $HgCl_2$ equals 0.033 mg SH). The accuracy of this technic was tested by titrating a standard solution of glutathione at frequent intervals.

Results. A summary of the mouse epidermis response to different polycyclic carcinogens topically applied is presented in Fig. 1. The changes observed in the dermis are in Fig. 2. The control values are indicated by the solid horizontal lines. (The figures represent the averages of the determinations at each time interval.)

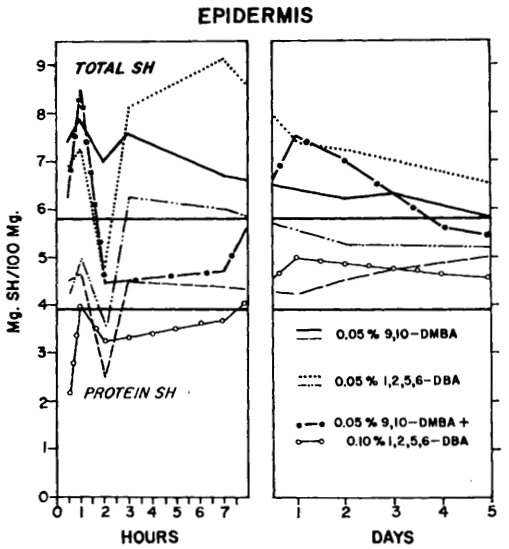

FIG. 1. Changes produced per 100 mg of mouse epidermis by one painting of a carcinogen or by a mixture of 2 carcinogens. Upper horizontal line is mean control value obtained for total sulfhydryl and lower horizontal line is mean control obtained for protein sulfhydryl.

At frequent intervals untreated mice whose dorsal hair had been clipped were used as controls for the epidermis and the dermis. The total sulfhydryl level of the epidermis and the dermis was 5.8± .57 mg/100 mg and 3.7± .47 mg/100 mg respectively; the protein sulfhydryl level of epidermis was 3.9 ± .57 mg/100 mg and that of the dermis was 2.4 ± .32 mg/100 mg. In both the epidermis and the dermis the protein sulfhydryl constitutes about 2/3 of the total sulfhydryl although it should be noted that concentration of total sulfhydryl in the dermis was about 2/3 as much as in the epidermis. By subtraction, the remaining 1/3 was considered soluble sulfhydryl. Painting the clipped skin of mice with the acetone solvent did not produce any change in the sulfhydryl levels measured.

Three compounds in acetone solution were tested for their effects on skin sulfhydryl levels. Two of these were the carcinogens DMBA and DBA. They were also tested in combination because DBA is known to inhibit skin carcinogenesis caused by DMBA(9). The last compound. anthracene, did not appear in either figure because the reactions to it were always within the untreated ranges. It was used as another control because it is a polycyclic hydrocarbon with no carcinogenic action on mouse skin(10).

The preliminary work on the soluble sulfhydryl level of whole skin demonstrated that a pronounced change occurred soon after painting. Consequently, in these experiments determinations were made at ½, 1, 2, 3, 7, and 24 hours and daily through 5 days.

Anthracene at double the concentration of the carcinogens used had no effect on either the sulfhydryl level of the epidermis or the dermis. The total sulfhydryl and the protein sulfhydryl levels were within the normal range at all intervals tested.

Epidermal changes. During the first hour total sulfhydryl content was above normal for all compounds tested (Fig. 1). The 0.05% DBA and the combination 0.5% DMBA-0.10% DBA caused a fall in total sulfhydryl content which reached its lowest point 2 hours after treatment. The low point in either case was about the same and averaged 4.5 mg/100 mg of epidermis or about 75% of the mean control value. At the third hour the level had begun to rise and individual differences became apparent. For instance, with DMBA, the total sulfhydryl level slowly returned to normal by the fifth day; the DBA reached a maximum of 9 mg of total sulfhydryl/100 mg of epidermis at 7 hours, slowly diminished and had not returned to normal at the fifth day. The most outstanding feature of the combina-

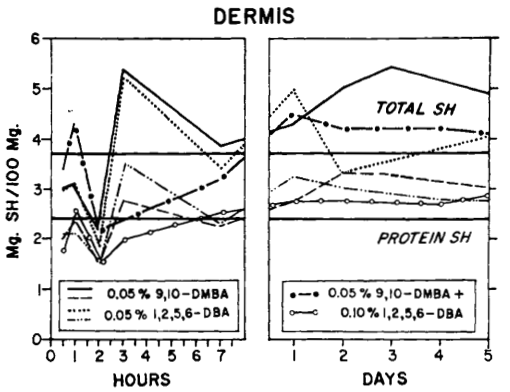

FIG. 2. Changes produced per 100 mg of mouse dermis by one painting of a carcinogen or by a mixture of 2 carcinogens. Upper horizontal line is mean control value obtained for total sulfhydryl and lower horizontal line is mean control obtained for protein sulfhydryl.

tion was the sustained decline for several hours. The effect on the protein sulfhydryl for the most part paralleled the corresponding total sulfhydryl curve with the following effect at the 2 hour levels: with DMBA, 2/3 of normal; with DBA barely below the mean control; with the combination, 15% below the mean control. In all 3 situations the protein sulfhydryl level was still above the mean control value at 5 days.

Dermal changes. In Fig. 2 are presented the changes which occurred in the dermis. At the first hour the total sulfhydryl was above normal with the combination of DMBA-DBA only; however, all 3 curves were depressed by the second hour. The individual carcinogens attained a maximum at 3 hours. The weaker carcinogen, DBA, had a second peak at 1 day while the stronger carcinogen had a second peak at 3 days. The combination of both carcinogens had only one peak. Its maximum was not as high as either carcinogen alone. The protein sulfhydryl results were lower but similar to those obtained for total sulfhydryl.

Soluble Sulfhydryl. The soluble sulfhydryl obtained from the total sulfhydryl and protein sulfhydryl reflected the same changes described above. For example, at the 2 hour level soluble sulfhydryl levels were down 1/3 for the mixture of 2 carcinogens and for the DBA while the strong carcinogen was 250% of normal. The drop in soluble sulfhydryl was greater in the dermis and was equal to 1/3 of the control in the case of DBA and 1/2 of the control in the other 2 series. The falls in total sulfhydryl and protein sulfhydryl in the dermis are so similar as to indicate that very little change occurs in the soluble sulfhydryl of the dermis. It must be concluded that most of the soluble sulfhydryl change takes place in the epidermis.

Discussion. The activity of the carcinogenic agents on the sulfhydryl level observed in these experiments with mouse skin is in agreement with those reported by Wood and Kraynek(11) on effect of 3,4 benzpyrene on the plasma sulfhydryl in the dog. The sustained drop in sulfhydryl produced by the combination of the 2 carcinogens cannot be attributed to the total concentration of hydrocarbons. When either carcinogen was used at a concentration of 0.15%, the response pattern was similar to that obtained with lower concentration. No explanation is offered for the difference in response obtained by DMBA and DBA. Using an indirect method of determining glutathione, Crabtree(12) was not able to demonstrate an effect on the soluble sulfhydryl level of skin by 3,4 benzpyrene and DBA. Nor was there any indication of utilization in the end product of metabolism. Crabtree did report that naphthalene, phenathrene, and to a slight extent anthracene, when given in large doses decreased tumor induction by 3,4 benzpyrene or DBA and caused a decrease in the SH content of skin followed by a prompt return to the normal level.

Many approaches have been used to elaborate the place of -SH groups in carcinogenesis and growth. As early as 1931 Reimann(13) noted that SH groups caused increased differentiation and organization of cells. He postulated that a qualitative change in chromosomal chemical groups which controls differentiation and organization must occur before malignancy. According to Davidson and Lawrie(14) chromosomes are poor in sulfhydryl groups and the oxidation of such groups might be responsible for somatic mutations concerned in the initiation of cancer(15).

A comprehensive study on the role of SH groups in both chemical carcinogenesis and anticarcinogenesis was made by Crabtree(3). He showed that a number of compounds which are known to react with -SH group containing cell constituents can act as anti-carcinogens. He has suggested that carcinogens become attached to cellular constituents of the skin of mice only in the presence of -SH groups and that many inhibitors of carcinogenesis cause local depletion of sulphur in the skin.

The observations reported here do not offer an adequate explanation of the mechanism involved. The possibility has been ruled out that the maximal dilution reported might be actually a dilution factor due to edema. The wetbacks of control animals and animals treated with carcinogen or inhibitor did not show any significant weight differences when sacrificed at two hours and dried to constant

weight at 105°C. There are, however, a number of problems to be considered. Perhaps the only effect on these agents is to speed the metabolites of the sulfhydryl group out of the tissue or to prevent the production of these sulfhydryl groups. This assumes a rather high rate of metabolism of sulfhydryl by the skin. The possibility that there is a binding of these agents with sulfhydryl groups must also be considered.

Summary. An amperometric titration method for determining sulfhydryl groups was applied to mouse skin. The total sulfhydryl, the protein sulfhydryl and by subtraction, the soluble sulfhydryl contents were determined after painting with anthracene, DBA, DMBA, or a combination of the 2 carcinogens. The anthracene and the solvent, acetone, had no effect on the sulfhydryl levels. In general the shape of the curve of the total sulfhydryl content of the epidermis and dermis was the same, although the dermis contained approximately half that of the epidermis. For the most part, the effect on the protein sulfhydryl paralleled the corresponding total sulfhydryl curve. Most of the change in the soluble sulfhydryl level is presumed to occur in the epidermis. No cause and effect relationship to carcinogenesis or anticarcinogenesis is supported by these observations on the sulfhydryl groups of the skin.

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Antifibromatogenic and Antihysterotrophic Activities of Synthetic Androgens (19-nor-methyltestosterone, 19-nor-testosterone phenlypropionate, Δ^1-testosterone and Δ^1-androstenedione).* (23448)

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Both the progestational and antiestrogenic (antifibromatogenic, antihysterotrophic, antiluteinizing) activities of 19-nor-progesterone are greatly superior to those of progesterone (1,2,3). The same is true for 19-nor-ethinyltestosterone(4,5,6,7). The progestational activity of 19-nor-methyltestosterone in women is considerable(8); it is active by oral administration(9). Various activities of 19-nor-testosterone and of its esters also are remarkable (10). Results with the antifibromatogenic and antihysterotrophic activities of 19-nor-methyltestosterone (nMT) and of 19-nor-testosterone phenylpropionate (nTpp) are given in the present paper. Results obtained with Δ^1 derivatives of androstene also have been included. The glucocorticoid activity of Δ^1-hydrocortisone is several times that of

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