In Vivo Electrometric Study of Carcinogenic Hydrocarbon Interaction with Mouse Epidermis

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Abstract □ A bioelectrometric technique was implemented in the study of 3-methylcholanthrene and benzene interaction with the epidermis of hairless mice. In contrast to previous studies of this nature, the experimental results were obtained entirely *in vivo* without causing injury to the mice. The results obtained are analogous to titration curves of amphoteric macromolecules. Inspection of the curves and their temporal variations following the topical application of the hydrocarbons revealed the induction of significant changes in the density of ionogenic groups affixed to the epidermal colloids. The carcinogenic 3-methylcholanthrene was observed to induce consistently a reduction of net cationic fixed-charge density attributable to the loss or discharge of basic nitrogenous groups titratable above, at least, pH 7.4. A discussion of some of the possible mechanisms of this observation is presented. The results of the present study indicate the bioelectrometric method to be complementary to the more direct experimental techniques commonly employed.

Keyphrases □ Carcinogenic hydrocarbon interaction—mouse epidermis □ Epidermis, mouse—3-methylcholanthrene, benzene □ 3-Methylcholanthrene, benzene effects—epidermal net charge density □ Bioelectrometric determination—epidermis–hydrocarbon interaction

The ability of hydrocarbons such as 3-methylcholanthrene (3-MC) to induce malignantization following topical application to skin has been well documented (1). It has been found generally that the greater the extent to which a hydrocarbon possessing a reactive, electron-rich, *K*-region binds to the skin, the greater is its carcinogenic potency (2–4). It has also been demonstrated that binding occurs to protein components of mouse skin (5). The extent of interaction of the hydrocarbons varies with time (2, 4–7), reaching a maximum for 3-MC at approximately 10 hr. (7). The binding of carcinogenic relative to inactive hydrocarbons has been found to be especially high for water-soluble proteins extracted from mouse skin found in a fraction termed "proteins I" (8, 9); the *in vivo* binding of hydrocarbons to nucleic acids appears to remain in some doubt (10). It has been concluded (11) that oxidative biotransformation of the *K*-region of the hydrocarbons yields dicarboxylic acids which bind protein through amide, imide, or ester linkages but most probably through the formation of amides.

The experimental methods employed in these previous investigations concerning the extent and nature of hydrocarbon interaction with mouse skin have been destructive; they necessitated the sacrifice of the animals, disintegration of the skin, and fractionation of its constituents. Such procedures are disadvantaged by the difficulty and necessity of correcting for any *in vitro* binding of the carcinogen which may occur during the extraction process. The observation of significant amounts of *in vitro* binding has led some authors (12) to conclude that carcinogens that were not bound in the intact tissue became bound in the process of disintegrating the tissue. The difficulty of distinguishing between binding sites active *in vivo* and those activated by the *in vitro* treatment of the tissue is readily apparent. Results obtained from the use of destructive methods have, therefore, been open to question with respect to their verity in representing actual *in vivo* conditions.

In the present study, a nondestructive bioelectrometric method, previously described (13, 14), was utilized. It permitted the investigation of hydrocarbon interaction with mouse epidermal colloids to be accomplished entirely *in vivo* under physiological conditions. The completely innocuous nature of the experimental procedure allows each animal to serve as its own control; repeated measurements can be made on the same animal to discern the temporal dependency of the induced effects. The present study was primarily undertaken to examine the ability of the bioelectrometric method to discern the effects of 3-MC on mouse skin and, secondarily, to determine if any responses to the carcinogen previously undetected when employing destructive techniques could be made apparent in an all *in vivo* study.

MATERIALS AND METHODS

Methods—The electrometric method employed in the present study is essentially based upon the Gibbs-Donnan equilibriumt It involves the measurement of electrical potentials developed at the boundary of the tissue and a buffer solution, which may or may not contain the substance whose interaction is to be studied. The results obtained from treated surfaces are compared to untreated controls.

The net density of fixed charge on the tissue surface, arising from the dissociation of ionogenic groups bonded or adsorbed to the surface, can be computed from the measured null point potentials. If these results are obtained under conditions of varying pH and plotted as a function of pH, the curves resemble, and are analogous to, titration curves of amphoteric macromolecules. The shape of these curves is dependent upon the nature of the dissociating groups, the presence of interacting solutes, and the state of aggregation of the colloids composing the tissue surface. The fixed-charge density on tissue surfaces and, therefore, the shape of the titration curves are quite sensitive to interacting substances. Variations in the curves can be interpreted to yield information about the extent and nature of the interaction.

The verity of the measured potentials in representing the fixed-charge density has been established in the principal author's laboratory (unpublished) as well as by others (15). The details of the calculation of the net fixed-charge densities and the elementary interpretation of titration curves have been described (13–15). It has recently been found, in the principal author's laboratory, that for human epidermis the thickness of the colloid phase, whose properties are reflected in the electrometric measurements, is approximately 4 μ. This represents about 40% of the depth of the stratum corneum. Although this same determination has not been performed for mouse skin, which is thinner by several cellular layers, the results of the present study could pertain, at least, to a similar relative depth.

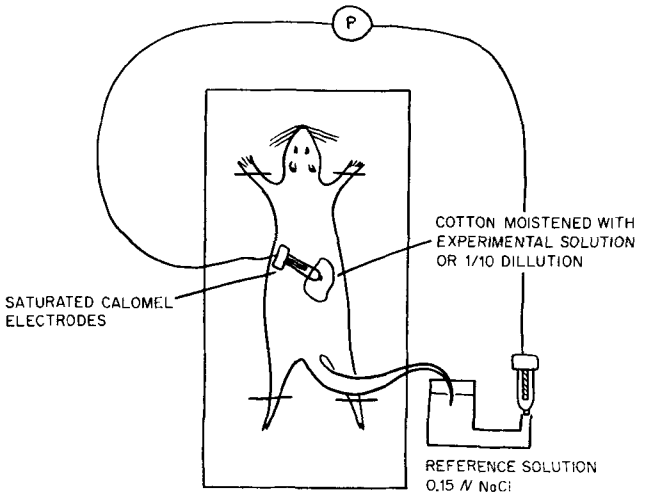

Figure 1—*Experimental arrangement for the determination of fixed-charge density of the epidermal surface of hairless mice.*

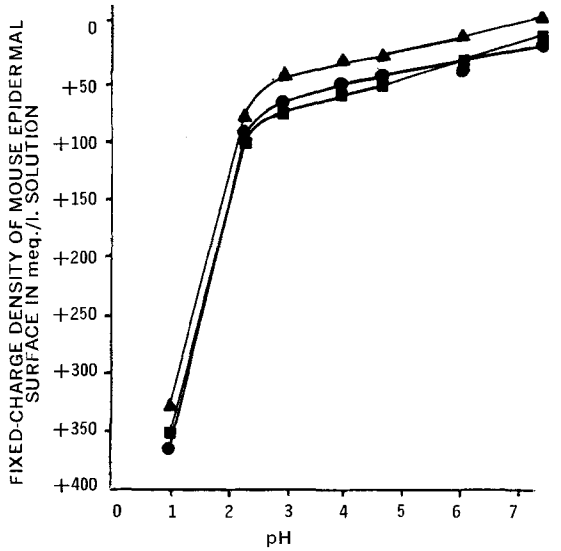

Figure 2—*The average relationship of the fixed-charge density of the epidermal surface colloids of mouse skin to pH. Each point represents the average of 24 determinations on four separate mice studied at each of six different time intervals varying from 1.1 to 50.3 hr. following treatment with benzene (—■—) and 1% 3-MC solution in benzene (—▲—). The remaining curve (—●—) represents results obtained from the untreated control skin areas on each of the mice.*

Figure 1 contains a diagram of the experimental arrangement used in the present investigation with hairless mice. The mice were ether-anesthetized and rendered immobile for the treatments and subsequent measurements by restraining them securely to a specially constructed mouse board prior to their recovery from the anesthetic. The differences of the measured potentials, used for the calculations of the fixed-charge density, are independent of the location of the reference surface on the animal. The tail of each mouse was selected for use in the present case because of its convenience. It was rinsed well with distilled water and 0.15 *N* NaCl and placed into the vessel containing the 0.15 *N* NaCl reference solution. The tail was not disturbed further during the course of the series of measurements corresponding to any given time. The dorsal side of the mouse was cleansed by swabbing with 70% ethanol, followed by a thorough, yet gentle, scrubbing with a solution of soap, and rinsing with distilled water. This cleansing procedure had previously been found to reduce both intrasubject and intersubject variations of the potential differences measured under any given set of conditions (13).

After cleansing, the back of the mouse between the neck and the tail was divided equally into three separate areas by drawing thin lines with an ink marker. The anterior third of the back was treated three times with a liberal application of benzene on a cotton swab and allowed to dry. The posterior third of the back was similarly treated with a 1% w/v solution of 3-MC in benzene. The middle area was left untreated and served as the control. Immediately prior to the measurement of potentials from any of the areas on the mouse, the area was rinsed with distilled water and allowed to hydrate by covering with a pledget of cotton wetted with 0.15 *N* NaCl for approximately 15 min. The electrical potentials were consistently read from the three areas in the order of benzene-treated, control, and 3-MC-treated skin. No significant differences in measurements performed on the three skin areas prior to their treatment were observed.

Titration curves for each treatment area on four separate mice corresponding to approximately 1, 4, 9, 20, 34, and 50 hr. after treatment were constructed over a range of pH from 1.0 to 7.4, using values of fixed-charge density calculated from the observed potential differences.

The data for a titration curve were obtained by swabbing the area with pH 1.0 buffer following the 15-min. soaking with 0.15 *N* NaCl. A 0.5 × 0.5-cm. piece of filter paper saturated with pH 1.0 buffer was placed in the center of the area of interest. Despite any movement by the mouse, the paper was held firmly in place, in part by the weight of the calomel electrode mounted in a movable arm assembly. A potential, E_1, was continuously recorded until observed variations in the potentials were less than approximately 0.2 mv./min.; this stability was usually achieved within 1–2 min. The electrode was then removed, the tip rinsed with saturated KCl solution, and wiped clean. Any remaining excess solution was swabbed from the back of the mouse. A smaller piece of filter paper, wetted with 0.15 *N* NaCl (except at pH 1.0 where a 1:10 dilution of the buffer itself was used), was placed within the area previously covered and was followed immediately by the replacement of the electrode and the recording of a potential, E_2. The electrode and skin surface were treated again as described, and the potential E_1 was redetermined to ensure that conditions had remained constant between the time of E_1 and E_2 measurements. The potential difference, $E_2 - E_1$, termed the dilution potential, E_d, was computed with the average of E_1 values which agreed within 1.0 mv. The E_d values allow the calculation of the fixed-charge density in the manner previously described (13, 14). Following pH 1.0, the fixed-charge densities of the epidermal surface corresponding to pH 2.2, 3.7, 4.6, 6.0, and 7.4 were obtained from dilution potentials measured in the same manner using the isoosmotic buffer solutions of higher pH.

Table I—Significance Levels Obtained by Analysis of Variance

Main Effects and Primary Interactions	*p*
Treatments of skin areas	0.05
Time	0.001
pH	0.001
Time–treatments	0.01
Treatment–pH	N.S.
pH–time	0.001

Materials—The compositions of the isoosmotic buffer solutions have been reported earlier except for the pH 1.0 solution which consisted of 0.10 *M* HCl and 0.05 *M* NaCl. Spectrograde benzene was used. The 3-MC was supplied by Eastman Chemicals.

Hairless, strain HRS/J mice (Jackson Labs, Bar Harbor, Maine) were used in the experiment. The mice were 7-week-old males.

Corning miniature fiber-junction saturated calomel reference electrodes were used in conjunction with a Sargent model SR potentiometric recorder. The potentials were read directly from the recorder chart.

Statistical calculations were conducted with the aid of an IBM 7094 computer.

RESULTS

Gross Graphical and Statistical Results—The experiment can be described as being of three-factor, unsymmetrical design. Four responses, corresponding to the replicate determinations of the dilution potential on four individual mice, were recorded for each factor level. The determinations were made at 1.1, 4.8, 9.4, 20.4, 34.0, and 50.3 hr. and at pH 1.0, 2.2, 2.9, 3.9, 4.6, 6.0, and 7.4 on each of the treated and control areas on the mice. Significance

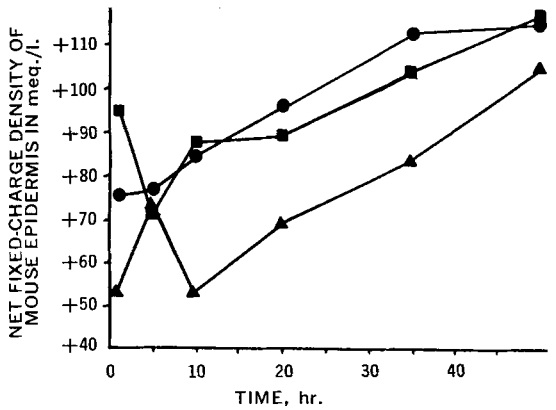

Figure 3—*The relationship of the net fixed-charge density of hairless mouse epidermis, averaged over pH, to the time following treatment of the skin with benzene (—■—), 1% 3-MC in benzene solution (—▲—), as well as for untreated control skin (—●—). Each point is the average of 28 determinations on four different mice.*

levels, resulting from an analysis of variance, for the main effects and primary interactions are shown in Table I; $p > 0.05$ was taken as not significant (N.S.). Some of the effects listed in Table I are graphically illustrated in Figs. 2–4. In Fig. 2, the fixed-charge densities plotted at each pH are averages of the values calculated from observed E_d values at each time and for each mouse. The relatively parallel nature of the titration curves corresponding to each treatment over considerable ranges of pH illustrates the insensitivity of the treatment effects to pH. The relatively small vertical distances between the curves and their crossing can account for the comparatively low order of significance of the treatments. Figure 3 is similar to Fig. 2, except the values of the fixed-charge densities are averaged over pH and plotted as a function of time. The pronounced effect of 3-MC, relative to the benzene control, is clearly evident for the majority of the time course of the study.

It is evident from Fig. 3 that a general trend toward an increased net density of positive fixed charge on the affected colloids underlies the treatment-induced responses. The three curves appear to possess this trend in common, differing only by the superimposition of the treatment-induced effects. Consistent with this trend toward a net increase in cationic fixed-charge density, examination of the titration curves for the untreated control skin areas revealed later curves

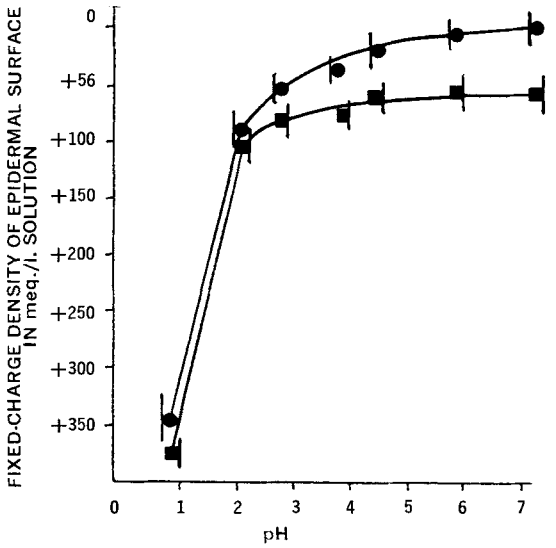

Figure 4—*Comparison of titration curves obtained for untreated mouse epidermis at 1.1 (—●—) and 50.3 (—■—) hr. Each point is the average of four determinations on separate mice plotted ± its standard error. By a paired t test, the curves are significantly different at* $p < 0.01$.

Table II—Statistical Probability Levels (p) Representing the Significance[a] of Observed Differences in Fixed-Charge Density of Benzene-Treated Mouse Epidermis, in Comparison to 1% 3-MC in Benzene Solution-Treated Mouse Skin as Determined from Paired t Tests

Time, hr.	pH 1.0	2.2	2.9	3.9	4.6	6.0	7.4
1.1	—	0.05	0.05	0.02	0.05	—	—
4.8	—	—	0.05	—	—	—	—
9.4	—	0.01	0.001	0.02	0.05	—	—
20.4	—	—	—	—	0.01	—	—
34.0	—	—	—	—	—	—	0.01
50.3	—	—	—	—	—	—	—

[a] Values of $p > 0.05$ were considered as insignificant and correspond to the blanks in the table.

always to lie below earlier curves. The extreme differences in the curves are depicted in Fig. 4 where the 1.1- and 50.3-hr. curves are presented. Each point is plotted with its standard deviation. A paired t test revealed the curves to be significantly different at $p < 0.01$. This agrees with the highly significant pH–time interaction and supports the reality of the underlying general trend toward positivity.

It can be surmised from further inspection of Fig. 3 that, relative to the benzene control, the treatment with 3-MC can be calculated from the vertical differences between the two curves to have induced an overall average reduction of 23% in net cationic fixed-charge density. Exempting the anomaly at 4.8 hr., the reduction appears to decline exponentially with time from 49% at 1.1 hr. to 10% at 50.3 hr. and has an approximate half-life of 20 hr.

To resolve more precisely the influence of pH and time on the effects of the benzene and 3-MC treatments, paired t tests were performed. The significance levels calculated from the tests are presented in Tables II–IV, from which it is evident that the effects are mostly significant at 1.1 and 9.4 hr. and nearly independent of pH. The highly significant differences between the 3-MC (plus benzene as the solvent) and benzene control can partly be attributed to the antagonistic effects of benzene and 3-MC on the net density of fixed charge at these times. This is evident from inspection of the titration curves observed at 1.1 and 9.4 hr. (Figs. 5 and 6) where it can be seen that the benzene and 3-MC displaced curves lie on opposite sides of the untreated control; moreover, in Fig. 3 the benzene curve is observed to lie above the untreated control at 1.1 and 9.4 hr. In each case a reduction of net cationic charge has been elicited by the 3-MC while the benzene has induced an increase at these times; the effect of benzene has subsided, while that of 3-MC has been elevated, at 9.4 hr. relative to 1.1 hr. The opposite effects of benzene and 3-MC are only apparent at 1.1 and 9.4 hr. At all other times the effects of the two hydrocarbons appear additive in reducing the average net density of fixed cationic charge on the involved colloids.

Superficially at least the relatively high significance of the effects of 3-MC at 9.4 and 1.1 hr. may be compared to the maximum in protein binding observed at approximately 10 hr. following treatment of mouse skin (7). That carcinogenic hydrocarbons interact with tissue constituents to alter cellular functions within an hour of their application to mouse skin has also been demonstrated (17, 18).

The appearance and disappearance of statistically significant treatment effects are likely the consequences of the various simul-

Table III—Statistical Levels of Significance[a] Resulting from Paired t Test Comparisons of Fixed-Charge Densities of Benzene-Treated Mouse Epidermis with Untreated Control Skin Areas on the Same Mice

Time, hr.	pH 1.0	2.2	2.9	3.9	4.6	6.0	7.4
1.1	—	0.01	0.05	0.01	0.01	—	—
4.8	—	—	—	—	—	—	0.05[b]
9.4	—	—	0.001	—	—	—	0.01
20.4	—	—	—	—	—	—	—
34.0	—	—	—	—	—	—	—
50.3	0.05[b]	—	—	—	—	—	—

[a] Values of $p > 0.05$ were assumed insignificant. [b] The benzene titration curve was observed to lie above the control.

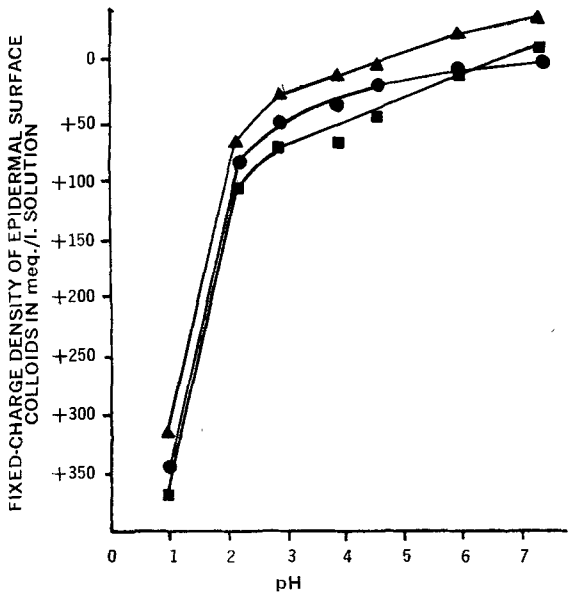

Figure 5—*Titration curves of hairless mouse epidermal surface observed at 1.1 hr. following treatment with benzene (—▲—), 1% 3-MC in benzene solution (—■—), as well as for untreated control skin areas (—●—) on the mice. Each point represents the average of four determinations on individual mice.*

taneously operative rate processes affecting the observed net density of fixed charge in a complex manner. In some instances, it is possible to resolve the influence of some of these processes.

DISCUSSION

Analysis of the Trend toward Positivity and the Time-Dependent Effects of Benzene—Close inspection of the untreated control titration curves in Fig. 4 reveals that the curves begin to diverge appreciably beyond pH 2.2, *i.e.*, in the range of pH where anionogenic groups generally become titratable. On this basis, the observed trend toward positivity can be attributed to a progressively increasing, time-dependent, net loss of anionogenic groups from the epidermal colloids. In contrast, a significant difference between titration curves

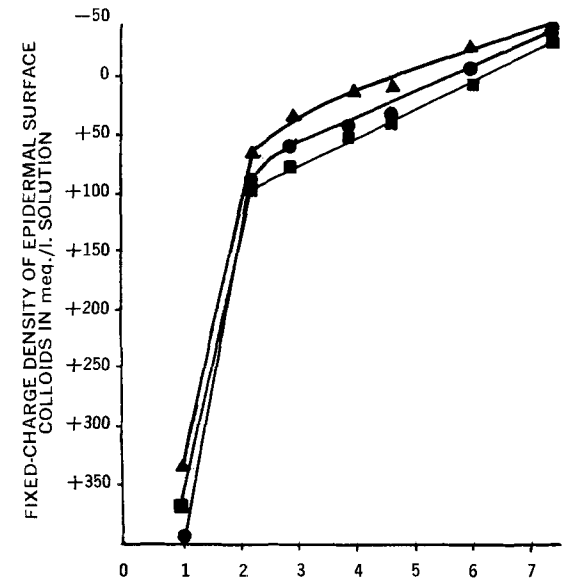

Figure 6—*Titration curves of hairless mouse epidermal surface observed at 9.4 hr. following treatment of the skin with benzene (—■—), 1% 3-MC solution in benzene (—▲—), as well as for untreated control skin (—●—) on the mice. Each point represents the average of four determinations on separate animals.*

Table IV—Statistical Levels of Significance[a] Resulting from Paired *t* Test Comparisons of Fixed-Charge Densities of Mouse Skin Treated with 1% Solution of 3-MC Relative to Untreated Control Skin on the Same Animals

Time, hr.	pH 1.0	2.2	2.9	3.9	4.6	6.0	7.4
1.1	—	—	—	—	—	—	—
4.8	—	—	0.02	—	—	—	—
9.4	—	0.05	0.01	0.01	0.01	0.05	—
20.4	—	—	—	0.05	0.05	0.01	0.02
34.0	—	—	—	—	0.001	—	0.02
50.3	—	—	—	—	—	—	—

[a] Values of $p > 0.05$ were assumed insignificant.

at pH 1.0, coupled with significant vertical differences at higher pH, characteristically accompany a diminution of cationogenic groups titratable in the pH range presently studied. In this case the curves would also be required to approach one another and eventually become coincident once the pH range in which these groups are titratable has been exceeded. Clearly this behavior is not characteristic of the curves in Fig. 4. The loss of anionogenic groups from the epidermal colloids remains the only acceptable explanation of their behavior.

Comparison of the benzene control with the untreated control curves (Figs. 5 and 6) observed at 1.1 and 9.4 hr. reveals a similar loss of anionogenic groups to be responsible for the benzene-induced effects on the fixed-charge densities of the epidermal colloids. An initial acceleration in the loss of anionogenic titratable groups in response to benzene treatment, followed by a slowing in the rate of subsequent loss, could account for the relatively high significant differences between the benzene and control curves at 1.1 hr. and the subsequent loss of significance at later times as the extent of anionogenic group loss from the untreated control skin approaches that for benzene-treated skin. Since carboxyl groups are primarily titratable over the pH range for which the benzene-control differences are significant, it may be speculated that the titratable anionogenic groups were initially diminished by the extraction of fatty acids in the benzene treatment and later lost through further elutriation into the applied 0.15 *N* NaCl and buffered titrating solutions. Joseph *et al.* (19) have made similar observations on toluene-treated skin and derived similar conclusions regarding the effect of the organic solvent.

It can be further hypothesized that the approach of the benzene curve in Fig. 3 to the control curve, in addition to being a consequence of the slowing of the benzene-induced initially accelerated loss of anionogenic materials, is also a result of a slower loss of cationogenic materials from the epidermis which becomes increasingly appreciable with time. Support for this hypothesis is gained from observing that beyond 1.1 hr., the benzene titration curve at pH 1.0 consistently lies above the control, even though the only individual difference that is significant occurs at 50.3 hr. It has been demonstrated (20) that the treatment of skin with organic solvents removes lipoidal substances but does not directly remove hydrophilic materials from skin. However, such treatment predisposes their loss from the skin by leaching when the skin is subsequently exposed to water or aqueous solutions. The loss of these hydrophilic materials is accompanied by a lowering in the water-sorption capacity of the skin. It has been shown that amino groups are predominantly responsible in affecting the water-binding capacity of skin (21). The water-soluble extractives from skin have been identified as nitrogenous and consisting of amino acids and polypeptides (20, 22). The pretreatment of the skin with 70% ethanol and soap may also be expected to have contributed to the elutriative loss of materials from the skin into the applied aqueous solutions. The untreated control skin, however, appears to have been left substantially unaffected with regard to changes in cationic fixed-charge density, as evidenced by the observation of the relatively constant values of positive fixed-charge density at pH 1.1 having a range of 350–380 meq./l. and an overall average of 361 meq./l. The higher values consistently correspond to later times.

In addition to this explication of the observed behavior of the control and benzene-displaced titration curves, the possibility of the changes resulting from the systemic absorption or lateral diffusion of 3-MC into the control areas also exists. To reduce the possibility of

systemic absorption in this study, the mice were isolated between measurements to prevent their licking one another. Iversen and Evensen (16) minimize the importance of systemic absorption of the hydrocarbons in affecting their results. Since each treatment area would be equally affected by systemically absorbed materials, the differences observed between the skin areas could still be attributed to the treatments, even if systemic absorption of the hydrocarbons occurred to an appreciable extent.

Reduction of Net Cationic Charge Density by 3-MC—The approximately constant vertical distance between the 3-MC and other curves in Figs. 2, 5, and 6 demonstrates that the 3-MC-induced diminution in net fixed-cationic charge density does not appreciably rely on pH in the range studied. Such pH independence may be expected to result only if the affected cationic groups are titratable in an alkaline range of pH above the limit of pH 7.4 adopted in the present study. Such basic groups could include the guanidyl group of arginine, the ϵ-amino group of lysine, α-amino groups (23), and some of the nitrogenous groups of purine and pyrimidine bases (24). It is, of course, not possible to discern the individual involvement of each of these groups in the presently observed phenomena. Provided the pK's of the different groups are sufficiently separated, further information might be gained in a future study by extending the alkaline limit of pH and noting the region of pH at which the anticipated coincidence of the 3-MC- and benzene-displaced titration curves is observed. Such observations might conceivably distinguish between the involvement of α-amino, ϵ-amino, and guanidyl groups.

It could be a temptation to conclude that the observed neutralization of charge on the involved base-binding groups, induced by the action of 3-MC, results from the acylation of these groups by carboxylated derivatives of the hydrocarbon. However, the observed up to nearly 50% involvement of the total fixed cationic groups on the colloids is quantitatively grossly inconsistent with the estimated 0.001% of amino acid residues which become conjugated with hydrocarbons (25). The discrepancy would not be appreciably mitigated even if the latter percentage is corrected on the basis of assuming that only strongly basic side-chain amino groups interact with the hydrocarbons. Assuming arginine and lysine to constitute 16.65% of the total amino acids in epidermis (26), a maximum of only 0.006% of their number would be directly bound to the hydrocarbons. Including the possibility of other binding sites as well, *e.g.*, as might occur on nucleic acids, would again diminish this value and add further to the disagreement. A greatly potentiated elutriative loss of water-soluble cationic constituents from the skin, induced by the 3-MC, is not considered a likely mechanism for the unaccounted changes since the observed reduction in net cationic charge, relative to benzene, is maximal at 1.1 hr. As previously discussed, the extent of such losses by leaching increases with time.

Although the results of the present study do not justify the establishment of any firm conclusions regarding the mechanisms by which the 3-MC-induced reduction of fixed cationic charge density is manifested, it is certainly interesting to speculate the possibilities. The simplest possibility to consider is for the interaction of carboxylated derivatives of 3-MC with the tissue colloids to occur through the action of secondary valence forces in addition to the formation of covalent linkages. The severity of the extractive procedures used in direct studies of hydrocarbon–protein interaction only allows the detection of the latter type of combinations. The use of the electrometric method permits changes in cationic fixed-charge density resulting from either type of binding to become demonstrative. The reversible nature of electrostatic and Van der Waals' binding could conceivably allow the resolution of the contribution of each type of interaction to the reduction of net cationic charge density to be elucidated. The removal of the labile interacted hydrocarbon from the skin might be accomplished through extensive washing of the surface. The decline of the 3-MC effect, relative to the benzene control, could have arisen through such a loss of weakly bound anionic derivatives of 3-MC into the applied solutions.

However, if the oxidative carboxylation of the hydrocarbon only occurs concomitantly with its covalent binding to protein (10), it then follows that free anionic forms of the hydrocarbon would not be available for secondary valence force interactions with the proteins. If this is the case, an alternative explanation of the observed diminution in cationic charge density has to be sought.

Because of its very extensive implications, an exceptionally intriguing possibility is the reduction of cationic charge density resulting from the operation of an allosteric mechanism (27). By this means, a cooperative release of protons from the affected macromolecular structures could conceivably result from the interaction of the hydrocarbon at a paucity of cardinal sites; these sites would, however, control the acid–base properties of comparatively large numbers of proton-binding sites. Consideration of Mason's bioelectronic theory (28) of carcinogenesis and Ling's association-induction theory (29, 30) lends some degree of credence to this conjecture. In the framework of Mason's theory, the release of protons from the macromolecular structures could result from the formation of mobile positive holes in the protein molecules following the fusion of conduction bands between the macromolecules and the interacted hydrocarbons. Inductive and electrostatic field effects responsible for the release of protons could be conveyed to the basic side chains in a manner analogous to the mechanisms postulated by Ling (29). A net reduction of cationic charge on macromolecules may be expected to diminish their affinity for forming polysalt complexes with polyanions, such as polynucleotides, or cause the dissociation of such complexes already in existence (31). Such hydrocarbon-induced reductions of cationic charge density on nuclear proteins could conceivably accomplish the derepression of genes central to Pitot and Heidelberger's theory of carcinogenesis (32).

This last hypothesis, in addition to being able to explicate the results observed in the present study, appears to be capable of integrating the various prominent theories of carcinogenesis. Although direct experimental evidence for the hypothesis is at present obviously lacking, its verity could readily be tested on the basis of gaining predictive evidence from both *in vivo* and *in vitro* experimentation. This and the determination of whether the effects of hydrocarbon–tissue interactions involving *K* and *L* regions of the molecules can be distinguished by the bioelectrometric method could provide worthwhile objectives for future experimentation.

CONCLUSIONS

Despite the apparent need for conjecture in some instances to interpret the results, the utility of the bioelectrometric method as a tool complementary to more direct, although destructive, techniques by which tissue–carcinogen interactions can be studied has been demonstrated in the present investigation. It would be of interest in future studies to prolong the experiment to determine whether the method is capable of predicting imminent neoplasia prior to the appearance of morphologic evidence.

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Acute Oral Toxicity of Dimethoate in Albino Rats Fed a Protein-Deficient Diet

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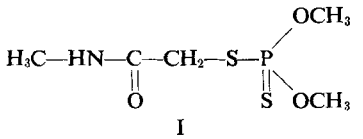
Abstract □ The oral $LD_{50} \pm SE$ of dimethoate was found to be 147 ± 29 mg./kg., 152 ± 22 mg./kg., and 358 ± 9 mg./kg., respectively, in male albino rats fed for 28 days from weaning on a diet containing: (*a*) 3.5% protein as casein, (*b*) normal amounts of protein as casein, and (*c*) normal amounts of protein as laboratory chow. The toxic syndrome at the range of the LD_{50} was similar in animals of all three dietary groups and consisted of signs of cholinergic stimulation, depression of the central nervous system, anorexia, hypothermia, aciduria, proteinuria, an irritant gastroenteritis, widespread vascular congestion, toxic degenerative changes, dehydration, and loss of weight in body organs.

Keyphrases □ Pesticide toxicity study—rats □ Dimethoate—toxicity study, rats □ Protein-deficiency effect—dimethoate toxicity □ LD_{50}—dimethoate □ Toxic effects, rats—dimethoate

Dimethoate, an organophosphorus pesticide introduced in 1956, is effective as a systemic insecticide against pests such as houseflies, aphids, mites, and grasshoppers (1). It is a white crystalline solid with limited solubility in water but is freely soluble in cottonseed oil. The formula is shown in Structure I. It is



I
dimethoate

absorbed into plants and converted mainly to the toxic oxygen analog (2) which is taken up by insects. Insects are very sensitive to its toxic effects (3), apparently due to less effective detoxifying mechanisms (4), particularly ability to remove the methylamido group which has been termed the "weak link" in detoxification by insects (5). Dimethoate is readily absorbed from the mammalian gastrointestinal tract and biotransformed by conversion of the sulfur group to the oxygen analog, by hydrolysis of the methyl ester groups, and by removal of the methylamido group (2). Pretreatment of mice with SKF 525A lessens (3), while pretreatment with the hepatic enzyme-inducer phenobarbital increases (6), the toxicity of dimethoate. The toxic action of dimethoate is due to inhibition of various body esterases (7). The acute oral LD_{50} of technical dimethoate in male rats has been reported to be between 180 and 325 mg./kg. (2). Tolerance in human foods has been set at 2 p.p.m. (8).

The World Health Organization concluded that further studies were desirable on the worldwide use of dimethoate (2) and requested the authors to investigate its acute oral toxicity in albino rats fed from weaning on diets deficient in protein. The present communication is a report of such studies.

EXPERIMENTAL

The experiments were performed on male albino rats of a Wistar strain.[1] Group I consisted of 137 weanlings, weighing 50–60 g., fed Protein Test Diet—Low[2] which contains 3.5% casein, 81.5% cornstarch, 8% hydrogenated cottonseed oil, 4% salt mix USP XIV, and 3% of an all vitamin mixture (9). A 4-week feeding period was selected from the studies of De Castro and Boyd (10) who found that weanling rats react rather violently to a protein-deficient diet during the first 2 weeks but adjust during the 3rd and 4th weeks. After 28 days of feeding, the animals weighed 50 ± 5 g. (mean ± *SD*).

Group II was a control group of 120 weanlings fed Protein Test Diet—Normal[2] which contains 26% casein, 59% cornstarch, and other ingredients as in Protein Test Diet—Low. At the end of 28 days, their body weight was 199 ± 18 g.

Group III was a protein control group of 128 rats purchased 2 weeks from weaning and fed a standard laboratory chow for 2 weeks.[3] This diet contains 24% protein from various plant and

[1] Obtained from Woodlyn Farms Limited, Guelph, Ontario.
[2] General Biochemicals, Chagrin Falls, Ohio.
[3] The chow was Rockland Rat Diet (Complete), Teklad Inc., Monmouth, Ill.