Interaction of Hexachlorophene with Human Epidermis II: Comparison of Kinetic and Equilibrium *In Vivo* Results Obtained by Direct and Bioelectrometric Methods

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Abstract □ The equilibrium uptake and release as well as the kinetics of hexachlorophene sorption and desorption on human epidermis were studied *in vivo*, utilizing a bioelectrometric technique and, more directly, a spectrophotometric assay for hexachlorophene cleared from and elutriated into aqueous solution. Kinetic and equilibrium results were obtained with 10 initial concentrations ranging from 0.116 to 1.26 mmoles/l. in a buffered medium at pH 10. Direct determinations of the uptake and subsequent studies of the release kinetics of hexachlorophene from the skin revealed that an average 41.6% of the hexachlorophene is recoverable by elutriation from the contents of three apparent kinetic compartments. Least-squares values of the half-lives of 1.42, 12.1, and 30.9 min. were found to correspond to each compartment. Wash-off experiments conducted for up to 400 min. revealed the operation of a fourth compartment with a half-life of approximately 274 min. Comparable kinetic and equilibrium results obtained electrometrically, in the form of the time course and equilibrium variation of the net fixed-charge density on the epidermal colloids, correlated with those obtained by direct assay for the slower compartments. The slower compartments were attributed to tissue-interacted hexachlorophene and the fastest compartment to its distribution into tissue-free space.

Keyphrases □ Hexachlorophene—equilibrium and kinetics of sorption–desorption on human epidermis, bioelectrometric determination, compared to spectrophotometric determination □ Epidermis, human—equilibrium and kinetics of hexachlorophene sorption–desorption, bioelectrometric determination, compared to spectrophotometric determination □ Bioelectrometric determination—equilibrium and kinetics of sorption–desorption, hexachlorophene on human epidermis, compared to spectrophotometric determination □ Sorption–desorption equilibrium and kinetics—hexachlorophene on human epidermis

Hexachlorophene is the active constituent of many substantively active antibacterial skin care products. To promote the effectiveness and minimize the toxicity of such products, it becomes important to elucidate the kinetics and mechanisms of the processes involved in the interaction of hexachlorophene with human skin. Following transient exposure of the skin to hexachlorophene, the substantive antibacterial behavior of hexachlorophene can readily be envisaged to depend upon the extent of hexachlorophene sorption onto epidermal colloids and the rate(s) at which it subsequently becomes available to affect microorganisms. Optimally, a maximum quantity of the agent would become sorbed very rapidly; following removal of the hexachlorophene reservoir contained in the vehicle in which it is applied, the residual sorbed hexachlorophene should become available at a rate sufficient to maintain an antibacterially effective level of the agent on the skin for a maximal period of time. Since these processes can be expected to be influenced by the other constituents of a product formulation, it is obviously of interest to quantitate and describe mechanistically the hexachlorophene–skin interaction behavior under controlled conditions in order subsequently to gauge and possibly predict the influence of adjuvants.

The first report (1) in the present series described the successful implementation of a bioelectrometric method to the elucidation of the influence of pH on the hexachlorophene-induced change in the net density of fixed charge residing on the colloids constituting the epidermal surface. A subsequent report provided confirmatory predictive evidence for the postulated mechanisms (2). The bioelectrometric method is advantaged by its rapidity, convenience, entirely innocuous nature, and ability to be applied under actual product use conditions using human subjects. As previously reported (1–6), the results obtained with this technique often can be interpreted to provide considerable insight into the molecular and bioelectronic mechanisms of the observed phenomena.

The purposes of the present study were: (*a*) to elucidate the dynamics of hexachlorophene–skin interaction behavior, and (*b*) to assess further the utility of the bioelectrometric technique in its application to the characterization of the kinetic and equilibrium sorption properties of hexachlorophene on human skin. These objectives were approached by designing experimentation that permitted a comparison of electrometric results with similar data derived more directly from the detection of hexachlorophene by spectrophotometric assay.

MATERIALS AND METHODS

Materials—The composition of the solutions and the apparatus used in this study were reported previously (1). The experiments were performed with 21–25-year-old human male volunteers.

Electrometric Monitoring of Hexachlorophene Sorption Kinetics—The time variation of hexachlorophene sorption onto human finger epidermis was followed through the implementation of an electrometric method; the method involves the measurement of potential differences, *i.e.*, dilution potentials, E_d, from which the fixed-charge density, f, of the tissue surface can be computed. The magnitude of observed changes in fixed-charge density reflect the extent of solute interaction with the tissue surface (4). The details of the experimental procedure for the measurement of E_d (1, 6), as well as the treatment and interpretation of results (1–6), were described previously. The verity of the quantitative representation of the fixed-charge density of colloid surfaces by measured values of E_d was confirmed by direct experimentation (7).

In the present work, hexachlorophene sorption was studied from 10 different pH 10 buffered hexachlorophene solutions with concentrations varying from 0.116 to 1.26 mmoles/l. A pH 10 medium was chosen because of the previously observed (1) sensitivity of the fixed-charge density of the epidermal surface in the vicinity of pH 10 and the observation that dilute dispersions of a commercial hexachlorophene-containing deodorant bath soap in deionized

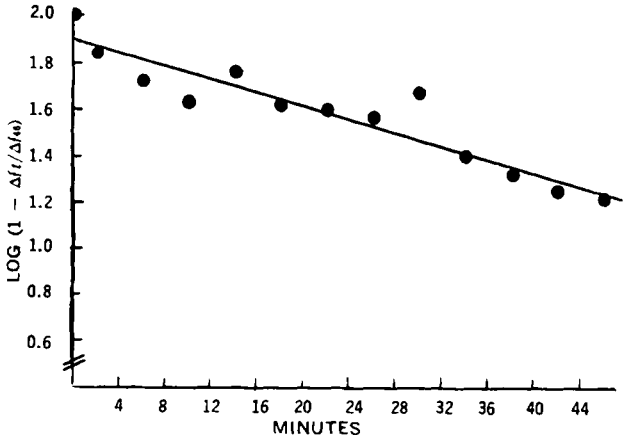


Figure 1—*Results of an electrometric study of the sorption kinetics of hexachlorophene onto human epidermis from a 0.340-mmole/l. hexachlorophene solution at pH 10.0;* Δf_t *is the observed change in the density of fixed charge at any time* t, *and* Δf_{46} *is the observed change after 46 min. Each point is the average of a minimum of four replications performed on each of two subjects.*

water possessed a pH of approximately 10. The composition of the buffer and the preparation of solutions were described earlier (1).

The time variation of hexachlorophene-induced changes in E_d was observed following the immersion of pretreated (1) fingers into a solution of hexachlorophene. Values of E_d were recorded at predetermined intervals up to 46 min.; it was found in preliminary studies that this length of time was necessary and sufficient for E_d values to become relatively constant, *i.e.*, vary less than 0.2 mv./min. A constancy in E_d was assumed to indicate the achievement of equilibrium. The observed values of E_d were transformed into the time course of change in fixed-charge density for each hexachlorophene solution by employing the alignment chart presented previously (1). The fixed-charge density of control fingers treated in an exact manner, except that they were soaked in the buffered vehicle without hexachlorophene, remained unchanged over the period of the sorption experiments.

Following the soaking-on of hexachlorophene, the solutions were spectrophotometrically assayed for remaining hexachlorophene. The differences from the total amounts initially present in the solutions are the total quantities sorbed onto the skin.

Spectrophotometric Determination of Hexachlorophene Desorption Dynamics—Immediately following the completion of the final potential measurement, the test fingers were removed from the hexachlorophene solution and any excess, clinging solution was blotted away. The fingers were then successively placed in contact with fixed volumes of 30 ml. of the pH 10 buffer solution for preselected time intervals, cumulatively totaling 68 min. One study was carried out with a 0.580-mmole/l. hexachlorophene solution to 400 min. Control fingers differing only in not being exposed to hexachlorophene at any time were treated in an identical manner. The solutions into which the hexachlorophene-equilibrated fingers were soaked were assayed spectrophotometrically for hexachlorophene, using the control finger solutions as blanks to correct for any material washed from the skin which could conceivably interfere with the absorbance readings.

The absorbance of the solutions was measured at 370 nm., which was found to be the wavelength of maximum absorbance. A spectrophotometer[1] with 1-cm. silica cells was used. A Beer's law plot was found to be linear over the concentration range of 0–0.12 mmole/l., using the pH 10 buffer as the vehicle; the molar absorptivity was 8.05×10^3. The overall average error of precision of the assay was 0.23%.

RESULTS

Electrometrically Studied Sorption Kinetics—The data obtained in the electrometric experiments were treated in a manner that allowed the results to be subsequently directly correlated with the results of the spectrophotometrically studied desorption kinetics. Values of $(f_t - f_\infty/f_0 - f_\infty)$ were calculated, where f_t is the fixed-charge density at any time t, f_0 is the fixed-charge density at zero time, and f_∞ is the asymptotic value of the fixed-charge density approached at or beyond 46 min. Semilogarithmic plots of $(f_t - f_\infty/f_0 - f_\infty)$ *versus* time, as exemplified in Fig. 1, were constructed for each hexachlorophene solution studied. For the majority of cases, the plots were quite linear. The plotted results are summarized in Table I by the listing of apparent first-order rate constants computed from the least-squares slopes of lines drawn through the points and the Pearson product-moment linear correlation coefficients. From these results, it appears that the kinetics of hexachlorophene-skin interactions, which are reflected in the electrometric results, can be described as apparent first order in most instances. However, the relatively low values of the linear correlation coefficient observed for some cases may be indicative of the operation of a more complex mechanism of interaction, even though the apparent independence of the rate constants on hexachlorophene composition further supports the assumption of first-order kinetics.

Table I—Summary of Half-Lives and Correlation Coefficients for Electrometric Monitoring of Hexachlorophene Sorption on Human Finger Epidermis

Initial Hexachlorophene Concentration, mmoles/l.	Half-Lives, min.	Correlation Coefficients
0.116	40.0	−0.850
0.235	16.7	−0.839
0.340	21.1	−0.931
0.448	23.5	−0.929
0.570	14.5	−0.898
0.700	18.7	−0.901
0.810	17.7	−0.865
0.975	15.9	−0.835
1.060	16.4	−0.759
1.260	14.8	−0.879

Spectrophotometric Study of Hexachlorophene Desorption—The cumulative amounts of hexachlorophene elutriatively recovered from the skin approached an asymptotic value in all cases. The cumulative amounts recovered were subtracted from the asymptotic values and were plotted as a function of cumulative soaking time on semilogarithmic coordinates; Fig. 2 represents a typical plot. These results were further analyzed by resolving the curves into a sum of contributing exponential terms as given by Eq. 1, where A_t is the quantity of hexachlorophene remaining to be desorbed, *i.e.*, the difference between the asymptotic value A_∞ and the cumula-

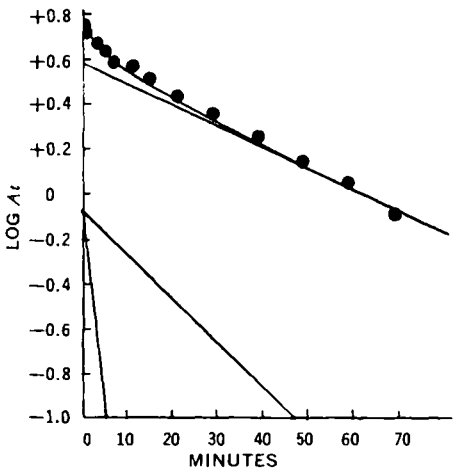


Figure 2—*Time variation of the logarithm of the quantity of hexachlorophene remaining to be released from human epidermis at pH 10 following soaking in a pH 10, 0.340-mmole/l. solution for 46 min. The lower plots represent a least-squares resolution of the desorption process into three exponential contributions. Each point is the average of four replications on each of two subjects.*

[1] Beckman DU.

Table II—Summary of Half-Lives for Each Compartment at Each Initial Hexachlorophene Concentration and Sum of Squares for Desorption Kinetic Data Which Has Been Converted to Sorption Values

Initial Hexachlorophene Concentration, mmoles/l.	Half-Lives, min., Compartment 1	2	3	Sum of Squares
0.116	28.6	8.4	8.30	0.0260
0.235	49.2	12.3	1.27	0.0658
0.340	31.4	14.4	1.50	0.0371
0.448	31.2	8.6	2.72	0.2061
0.570	32.8	9.6	1.21	0.1271
0.700	20.4	16.4	1.14	0.5636
0.810	19.3	15.9	1.00	0.5565
0.975	31.6	14.8	1.13	0.5459
1.060	21.2	43.0	1.81	0.4147
1.260	38.8	8.1	0.97	0.7901

tive quantity recovered at any time:

$$A_t = A_1^0 e^{-K_1 t} + A_2^0 e^{-K_2 t} + A_3^0 e^{-K_3 t} \qquad \text{(Eq. 1)}$$

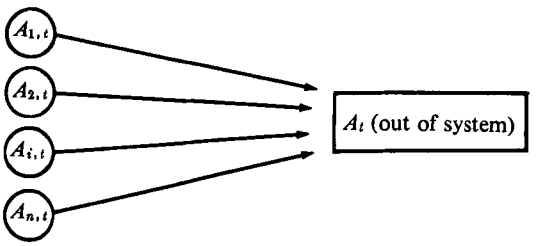
Values of the apparent transfer rate constants (K_1, K_2, and K_3) and intercepts (A_1^0, A_2^0, and A_3^0) were initially estimated using a computerized "peeling-off technique" (8–10), which allows a selection of the number of exponential terms (up to four) that are best included in the summation. Refined values of these parameters were obtained using a computerized iterative search convergence technique (9, 10), similar to the systematized iterative guessing method described by Ackerman and Hazelrig (11) which yields the weighted least sum of squares best fit to the experimental data. Values of half-lives were calculated as $t_{1/2} = 0.693/K_i$ for each value of K_i. These values, as well as the sum of squares corresponding to each multiexponential fit to the data, are presented in Table II. Figure 2 is representative of the graphical results. The upper curve shows the experimental points scattered around the least-squares multiexponential curve; the curve is the sum of the three linear exponential contributions, plotted in the figure, into which the curve was resolved. The hexachlorophene wash-off experiment, which was extended to 400 min., revealed an additional exponential contribution having a half-life of 274 min. In view of the numerous reports of substantive antibacterial effects (12–14), a slow release of residual hexachlorophene from the skin is not unexpected. Inspection of Table II reveals no general pattern of dependency of the apparent hexachlorophene desorption half-lives on the hexachlorophene concentration in the soaking-on solutions.

Equilibrium Sorption—The total uptake and recovery equilibrium quantities of hexachlorophene are listed in Table III along with the

Table III—Equilibrium Results of Hexachlorophene Uptake and Release from Human Finger Epidermis[a]

Hexachlorophene Concentration, mmoles/l.	Total[b] Hexachlorophene Sorbed, mmoles/cm.^{2} × 10^5	Total[c] Hexachlorophene Recovered, mmoles/cm.^{2} × 10^5	Fixed-Charge Density, meq./l.	Intercepts[d] in mmoles/cm.^{2} × 10^5 A_1^0	A_2^0	A_3^0
0	—	—	−323 ± 17	—	—	—
0.116	5.36	2.85	−283 ± 21	2.18	0.57	0.08
0.235	15.00	5.71	−270 ± 66	3.95	1.06	0.70
0.340	14.30	5.72	−262 ± 29	3.80	0.94	0.94
0.448	22.00	9.52	−258 ± 24	5.80	3.20	0.52
0.570	30.00	9.62	−251 ± 33	6.17	2.43	1.00
0.700	45.60	11.90	−270 ± 20	8.00	2.70	1.25
0.810	43.70	11.90	−253 ± 26	8.60	2.10	1.25
0.975	50.60	18.90	−252 ± 10	13.50	4.15	1.00
1.06	51.40	20.00	−253 ± 14	14.78	2.76	2.17
1.26	56.20	20.00	−246 ± 16	15.00	3.40	1.51

[a] Each value is the average of a minimum of four determinations on two subjects. [b] Quantities of hexachlorophene cleared from solution in 46 min./cm.^{2} of exposed finger. [c] Total quantities of hexachlorophene elutriatively recovered in 68 min. [d] Intercepts for each log-linear component of weighted least-squares fits to the time course of elutriative removal of residual hexachlorophene from finger epidermis.



Scheme I

equilibrium electrometric results. The total amount of hexachlorophene elutriatively recoverable in a wash-off experiment is given by the sum of the intercepts, *i.e.*, $\sum_{i=1}^{n} A_i^0$, of semilogarithmic plots of the exponential terms contributing to describe the time variation of the total residual hexachlorophene (*e.g.*, see Fig. 2). The A_i^0 values are listed in Table III. A linear least-squares regression plot of the total amounts of hexachlorophene detected to be elutriatively recoverable in the 68-min. wash-off experiments as a function of the total quantities cleared from the hexachlorophene solutions during the 46-min. soaking-on period provided a least-squares slope forced through the origin of 0.416. The linear correlation coefficient is 0.91, which is significant at $p < 0.01$. The slope of the curve indicates that an average 41.6% of the hexachlorophene taken up by the skin was recovered in 68 min. Increasing the desorption time to 400 min. increased the relative amount recovered to 55.0%. The remaining 45.0% may be assumed to be irretrievable or recoverable only very slowly.

DISCUSSION

Relationship between Electrometrically Monitored Sorption and Spectrophotometrically Determined Desorption—The wash off of hexachlorophene from the skin can be described as simultaneously occurring from a number of compartments into which it had originally distributed. Physically these compartments may be comprised of: (*a*) hexachlorophene in residual, clinging hexachlorophene solution and hexachlorophene solution that is absorbed into free tissue space such as is contained in the spongy keratin matrix of the skin; (*b*) hexachlorophene distributed into water of hydration, *i.e.*, water interacted with the epidermal colloids; (*c*) hexachlorophene distributed into richly lipoidal regions; and (*d*) hexachlorophene adsorbed onto any number of heterogeneous groups of tissue binding sites.

The compartmented system may be represented as shown in Scheme I, with one equation of the type $A_{i,t} = A_i^0 e^{-K_i t}$ appropriate for each route of loss; $A_{i,t}$ is the quantity of hexachlorophene remaining in the ith compartment at time t, and A_i^0 is the total equilibrium quantity which may be expected to depend upon the concentration of the soaking-in solution, the distribution coefficient(s), and the volume of the compartment, as well as the density and affinity of any binding sites. Scheme I obviously assumes that the release from each compartment behaves in at least a piecewise linear manner over the range of hexachlorophene content encountered with each hexachlorophene wash-on solution. The accuracy of this assumption may be expected to diminish with the concentration of the soaking-on solutions as the capacities of interaction sites tend to become saturated. That this is the case is indicated by the increase in the values of the least sums of squares of the multiexponential fits which occurs with increasing concentrations; the values are listed in Table II. However, inspection of the theoretical multiexponential fits to the data revealed them in each case to be satisfactory representations of the experimental points.

In accordance with Scheme I, the fractional content of a compartment remaining at any time following the initiation of a wash-out experiment is given by Eq. 2:

$$\frac{A_{i,t}}{A_i^0} = e^{-K_i t} \qquad \text{(Eq. 2)}$$

The fraction of the quantity of solute ultimately sorbed into a compartment at any time following the initiation of an uptake experiment is given by Eq. 3; the equilibrium content of the com-

Table IV—Summary of Correlation Coefficients for Direct (Converted from Desorption) and Electrometric Sorption Kinetics in the Two Major Contributing Compartments

Initial Hexachlorophene Concentration, mmoles/l.	Correlation Coefficient—Compartment—	
	1	2
0.116	0.0537	0.9410
0.235	0.3937	0.9960
0.340	0.9985	0.9964
0.448	0.9996	0.6823
0.570	0.9911	0.9802
0.700	0.9994	0.9988
0.810	0.9946	0.9904
0.975	0.9029	0.8523
1.060	0.7393	0.9303
1.260	0.1315	0.9760

partment, *i.e.*, at $t = \infty$, in an uptake experiment is assumed equal to the content of the compartment at $t = 0$ in the wash-out experiment:

$$\frac{A_{i,t}}{A_i^0} = 1 - e^{-K_i t} \qquad \text{(Eq. 3)}$$

Subtraction of $A_{i,t}/A_i^0$, corresponding to any time following the initiation of an uptake experiment, from unity provides the theoretical fractional content of the compartment at the same time following the initiation of a wash-off study. By assuming that the content of one or more compartments during the uptake study is directly related to the change in the fixed-charge density (Δf_t) of the epidermal surface colloids as determined electrometrically, the fractional content of the compartment is given by $\Delta f_t/\Delta f_\infty$. The quantity $1 - (\Delta f_t/\Delta f_\infty)$ obtained in the electrometric uptake experiments may, therefore, be directly compared to $A_{i,t}/A_i^0$ values obtained in the spectrophotometric wash-off studies. The results of such comparisons are presented in Table IV; Pearson r values are listed for each hexachlorophene soaking-on solution. These values reflect the goodness of a linear correlation betweeen $1 - (\Delta f_t/\Delta f_\infty)$ and $A_{i,t}/A_i^0$. The linear correlation coefficients are presented only for the two slower compartments detected in the wash-off studies. The fastest compartment, Compartment 3, was not included because its contribution to the overall sum of exponentials, *i.e.*, the total wash off, is negligible after about 10 min. whereas fixed-charge density changes in every case occur up to nearly 46 min.

The low values for Compartment 1 at the two lowest initial hexachlorophene concentrations may be due to the very small amount of hexachlorophene taken up becoming distributed almost entirely into Compartment 2. Except for Compartment 1 at the two highest hexachlorophene concentrations and Compartment 2 at 0.448 mmole/l., all other compartments showed a high degree of correlation with electrometric results. With the exceptions noted, it can be surmised that the hexachlorophene contained in both of the compartments is responsible for changes in the fixed-charge density of the epidermal surface. Due to the extremely rapid removal of hexachlorophene from Compartment 3, it is likely that this compartment consists of tissue-free space, which may be considered similarly to extracellular space (15). It is apparent that the drug which is distributed into this compartment does not make a significant contribution to changes in the fixed-charge density but may be in equilibrium with tissue-bound fractions of hexachlorophene, which are more directly responsible in effecting the observed changes. The apparent volume of the epidermal tissue-free space can be readily estimated as approximately 3.4% of the volume of the immersed fingers. The inordinately large magnitude of this value indicates it to be a solubility volume (8) in contrast to representing an actual physical space; the magnitude of the value is then attributable to a tissue space/external solution partition coefficient which is greater than unity. A value of 4%, obtained by an entirely different method, was reported previously (4).

Equilibrium Sorption Characteristics—The total change in fixed-charge density at equilibrium and the total amount of hexachlorophene estimated as bound onto the epidermal colloids are compared in Fig. 3. The bound hexachlorophene can be estimated as

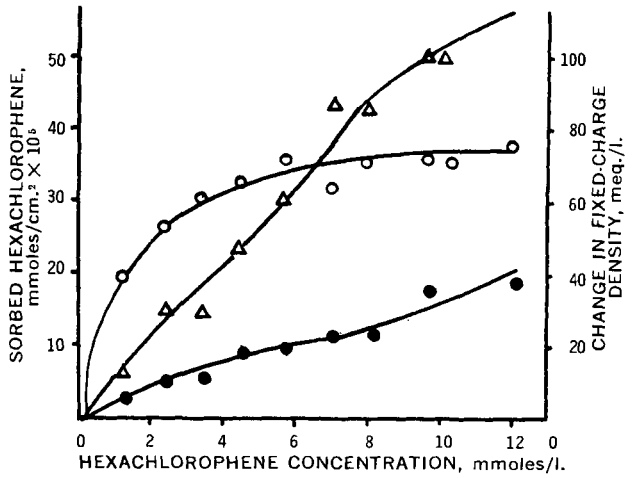


Figure 3—*Dependency of hexachlorophene-induced changes—in fixed-charge density* (○) *and quantity of hexachlorophene sorbed from solution in 46 min.* (△) *and elutriatively recoverable in 68 min.* (●) *from human finger skin—on the external concentration of hexachlorophene.*

the sum of the total amount of hexachlorophene contained in the slowest two compartments, *i.e.*, the total recovered corrected for tissue-free space or, alternatively, the total amount cleared from the soaking-in solutions. Both results are plotted in Fig. 3 as a function of the equilibrium free hexachlorophene concentration in the solutions. Linear correlations between the direct and electrometric results compared at the same hexachlorophene concentrations indicated that they were in each case significantly the same at the $p < 0.05$ level. From the somewhat hyperbolic shape of the curves in Fig. 3, it may be speculated that the binding of hexachlorophene to the skin exhibits Langmuirian-type behavior; *i.e.*, hexachlorophene is bound to independent, equivalent sites. This type of binding was reported by Breuer (16–18) for various phenolic compounds interacting with human hair.

SUMMARY AND CONCLUSIONS

The dynamic and equilibrium interaction behavior of hexachlorophene with human epidermis was studied electrometrically and, more directly, by employing a spectrophotometric assay. A comparison of the results showed the two methods to be in good correlative agreement. The electrometric procedure is advantaged by its relative convenience and rapidity as well as its capability to reflect selectively the quantity of hexachlorophene on the skin that resides in the slower compartments and likely exists bound to the epidermal colloids. These compartments contain the fractions of the total residual hexachlorophene, which is recoverable, and can be expected to be predominantly responsible for any substantive antibacterial activity of hexachlorophene on the skin.

The compartment having an average half-life of hexachlorophene release of approximately 36 min. contains the largest relative quantity that can be detected to be recoverable within 400 min. Provided the kinetics are not affected by other components of a product formulation, the quantities contained in the faster compartments are considerably less; in a practical skin-cleansing situation with an antibacterial soap, for example, the contents of the faster compartments may be appreciably deleted by loss into the rinse water alone. The slower compartment, having a half-life of 274 min., contains relatively little hexachlorophene and provides it at a rate that may be anticipated as too slow to make an appreciable contribution to the free, antibacterially active, hexachlorophene concentration present on the skin following its transient exposure to hexachlorophene onto the skin surface. Any slower compartments, if they exist, could be expected to be of even less significance. Even if it is assumed that the diffusive transfer of hexachlorophene within and from the skin might be unidirectionally favored to the exterior, the slower the rates of desorption of hexachlorophene the greater is the expectation that the hexachlorophene which is not elutriatively recovered becomes irretrievably lost through percutaneous absorption.

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Oral and Parenteral Formulations of Marijuana Constituents

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Abstract □ The lack of detailed information on the manipulation and preparation of cannabinoid formulations prompted an investigation of useful vehicles for administration of tetrahydrocannabinols and crude marijuana extracts. It was found that pure Δ^9- and Δ^8-tetrahydrocannabinols could be quantitatively handled by chipping samples at 4°, transferring them to cold receptacles for weighing, and, after liquefying the cannabinoid at 50°, adding a warmed vehicle for further transfers and final dilution. Tetrahydrocannabinol samples larger than 10 g. were liquefied at 55° and poured directly into a tared receptacle. Crude marijuana extract samples were smeared on tared receptacles and diluted and transferred as above. Stock solutions of cannabinoid in sesame oil were stable for months and could be used directly for oral administration or for formulating injectables. Suitable emulsions for parenteral use consisted of sesame oil (10–15%) plus polysorbate 80 (0.4–1%) in saline containing up to 4% tetrahydrocannabinol or sesame oil (5–10%) plus polyvinylpyrrolidone (4–5%) containing approximately 1% cannabinoid. Such an approach incorporated an innocuous vehicle, did not require the presence or removal of an organic solvent, provided wide latitude for needed concentrations of cannabinoid, and was timesaving. The ratio of emulsifier and cannabinoid was critical for stable emulsions.

Keyphrases □ Marijuana constituents—oral and parenteral formulations, stability in various solvents, biological evaluation of formulations, vehicle toxicity □ Toxicity, vehicle—stability of marijuana constituents in various solvents, biological evaluation of formulations for long-term oral and parenteral administration to laboratory animals □ Formulations—effect of solvents on marijuana constituent stability, biological evaluation, vehicle toxicity □ Tetrahydrocannabinols—stability in various solvents, biological evaluation of formulations for long-term oral and parenteral administration, vehicle toxicity

A full understanding of the biological properties of marijuana constituents has been hampered by the lack of a desirable vehicle for the preparation of injectables. The diversity of formulations and routes of administration of the active ingredients of marijuana has somewhat complicated the comparison of pharmacological, toxicological, and behavioral data from different laboratories. Those studies involving a single administration have justifiably not been too concerned with the influence of the vehicle. However, chronic investigations at relatively high doses (>50 mg./kg.) of cannabinoids cannot overlook the effect of the diluent.

The tetrahydrocannabinols have been identified as the major biologically active components of marijuana (1, 2). Whereas crude marijuana extracts are of a tar consistency, the tetrahydrocannabinols are highly viscous oils, virtually of a glue nature at room temperature. Despite reports of extensive analytical data to establish the purity of the sample, little detail has been given as to how to transfer and manipulate the compounds to obtain accurate concentrations.

Relatively high concentrations of tetrahydrocannabinol or crude marijuana extract for intragastric use can be achieved in natural vegetable oils (3). On the