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ABSORPTION OF SOME ORGANIC COMPOUNDS THROUGH THE SKIN IN MAN*

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ABSTRACT

We studied the percutaneous penetration of 21 organic chemicals. The experimental method consisted of the application of the chemical to the human forearm and quantitating its penetration through the skin by its appearance in urine.

There was a great diversity in the ability of the chemicals to penetrate human skin. Compounds such as hippuric acid, nicotinic acid, and nitrobenzene support the generally held view of the excellent chemical barrier properties for them.

Closely related compounds showed great differences in penetration. Benzoic acid was absorbed at 200 times the amount of its glycine conjugate—hippuric acid. Nicotinic acid barely penetrated; 10% of its amide, nicotinamide, penetrated. This suggests that molecules may be tailored to decrease or increase penetration as needed for the most suitable biological function.

We previously characterized the percutaneous penetration of a series of steroids in man (1). This study extends these observations using the same experimental method to several other simple organic chemicals. The method consists of the application of the chemical to the human forearm and quantitating its penetration through the skin by measuring its metabolites in urine. For analytic convenience, all studies were performed with radiolabeled (^{14}C) tracer doses. Measurement was made of the isotope only.

This study demonstrates diverse resistance to the penetration of these chemicals. Compounds such as hippuric acid, nicotinic acid and nitrobenzene support the generally held view of the effective barrier properties of the skin; others such as dinitrochlorobenzene (DNCB), caffeine and benzoic acid penetrate so extensively as to suggest that the human skin has little barrier properties to them.

METHODS AND MATERIALS

Methods of application of the chemical to the skin and analysis of radioactivity are those reported in the study of steroid hormones (1, 2). A compound to be studied must have a significant part of its metabolites excreted in urine. By using carbon^{14} labeled tracers, it is possible to measure excretion rates in urine without identification and measurement of individual metabolites. Correction for incomplete renal excretion must be made. Selected compounds were parenterally administered and the proportion of the carbon^{14} recoverable in urine determined. Ten compounds were thus given intravenously in man. Three possibly toxic compounds were given intraperitoneally to guinea pigs. Compounds not tested for incomplete urine excretion are closely related to compounds tested or are known to be nearly entirely excreted in urine.

In all experiments the amount of chemical applied was 4 micrograms/cm^2. The anatomic site was the ventral surface of the forearm. For accurate measurement, the chemical was dissolved in acetone and applied to a marked area with a microliter syringe. The standard method was to apply a 4 micrograms/cm^2 dose with one microcurie to a 13 cm^2 circular area. When the specific radioactivity of the chemical was too low to obtain the desired chemical concentration, the area was increased. Where the specific activity was high, carrier compound was added.

The skin sites were not protected. The subjects were asked not to wash the area for 24 hours. All urine was collected for 5 days. The carbon^{14} was measured in individual specimens by a method previously described (2). This consisted of wet ashing 5 ml of urine with subsequent trappings of the $C^{14}O_2$ with ethanolamine. This was added to a scintillator fluid and counted with appropriate standards in a Beckman liquid scintillation counter.

RESULTS

Excretion kinetics (Intravenous controls). Table I shows the total excretion of 10 com-

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TABLE I

^{14}C recovery in urine after i.v. administration

Compound	% Recovered	Half life
Caffeine	59.4	6 hrs.
Chloramphenicol	67.4	6 hrs.
Colchicine	27.9	4 hrs.
Diethyltoluamide	52.3	4 hrs.
Dinitrochlorobenzene	64.0	4 hrs.
Hexachlorophene	4.4	48 hrs.
Nitrobenzene	60.5	20 hrs.
Potassium thiocyanate	10.2	12 hrs.
Salicylic acid	89.8	4 hrs.
Urea	71.7	8 hrs.

TABLE II

^{14}C recovery in urine after i.v. administration in guinea pigs

Compound	% Recovered	Half life
Butter yellow	58.6	6 hrs.
Malathion	76.0	4 hrs.
Methylcholanthrene	18.2	14 hrs.

pounds after intravenous administration in man.

Three compounds, possibly too toxic to test intravenously in man, were administered intraperitoneally to guinea pigs. The results are in Table II. Nicotinic acid, nicotinamide, hippuric acid and phenol were assumed to have a similar renal excretion pattern to salicylic acid. Thiourea was assumed to behave like urea.

Topical administration. Table III gives the C^{14} recovery rate data after topical administration for each time period and the number of subjects, total recovery (in % of the applied dose) and standard deviations.

Figure 1 gives the total 5 day absorption expressed as % of the applied dose. These are listed in order of magnitude. Absorption of greater than 40% was noted with DNCB, caffeine and benzoic acid. Nicotinic acid, hippuric acid, and thiourea penetrated in a quantity less than 1% of the applied dose. The rate of penetration differed in each collection

TABLE III

Absorption after topical administration

Steroid	Absorption rate (% Dose/hr.) Time (hrs)						Total absorption		
	0–12	12–24	24–48	48–72	72–96	96–120	% of dose	S.D.	# of subj.
Acetylsalicylic acid	.141	.438	.334	.147	.076	.060	21.81	3.11	3
Benzoic acid	3.036	.340	.055	.000	.000	.000	42.62	16.45	6
Butter yellow	.215	.685	.289	.083	.054	.022	21.57	4.88	4
Caffeine	.559	1.384	.855	.109	.032	.014	47.56	20.99	12
Chloramphenicol	.007	.019	.021	.022	.015	.012	2.04	2.46	6
Colchicine	.036	.038	.033	.040	.025	.004	3.69	2.50	6
Dinitrochlorobenzene	3.450	.565	.134	.045	.018	.009	53.14	12.41	4
Diethyltoluamide	.773	.331	.084	.036	.016	.012	16.71	5.10	4
Hexachlorophene	.029	.031	.020	.028	.034	.030	3.10	1.09	7
Hippuric acid	.005	.003	.001	.001	.001	.001	.21	.09	7
Malathion	.313	.170	.044	.017	.011	.006	7.84	2.71	7
Methylcholanthrene	.062	.329	.258	.127	.064	.045	16.81	5.16	3
Nicotinic acid	.000	.002	.001	.001	.002	.007	.34	.09	3
Nicotinamide	.019	.168	.177	.088	.052	.031	11.08	6.17	7
Nitrobenzene	.022	.022	.013	.013	.011	.006	1.53	.84	6
Paraaminobenzoic acid	.159	.648	.444	.196	.058	.044	28.37	2.43	13
Phenol	.254	.091	.010	.601	.000	.000	4.40	2.43	3
Potassium thiocyanate	.051	.060	.078	.097	.100	.093	10.15	6.60	6
Salicylic acid	.116	.535	.356	.156	.080	.033	22.78	13.25	17
Thiourea	.046	.035	.010	.008	.007	.007	.88	.22	3
Urea	.008	.021	.051	.073	.075	.034	5.99	1.91	4

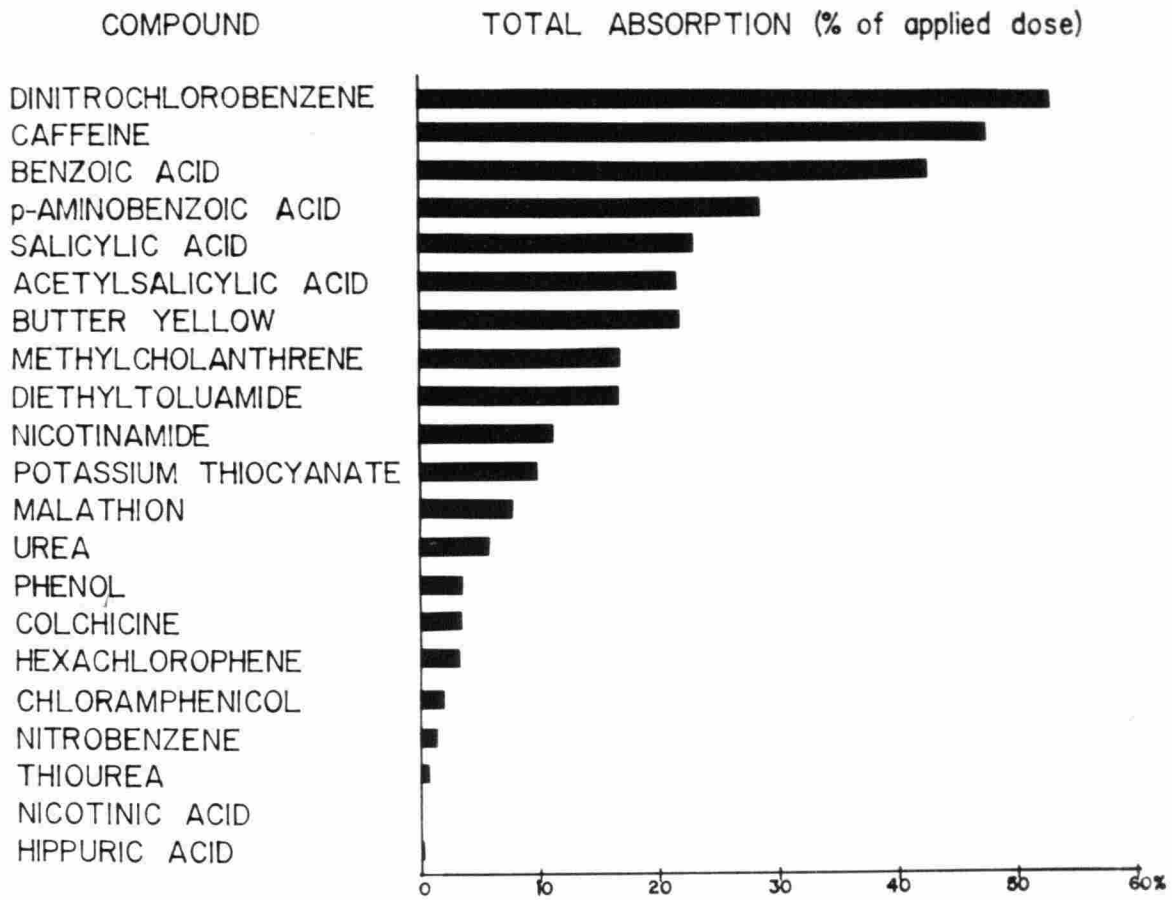

FIG. 1. The total absorption of % in applied dose. The forearm was the test site. These data represent the amount of isotope present in urine in the five days after cutaneous application. The applied dose was 4 μgm/cm^2.

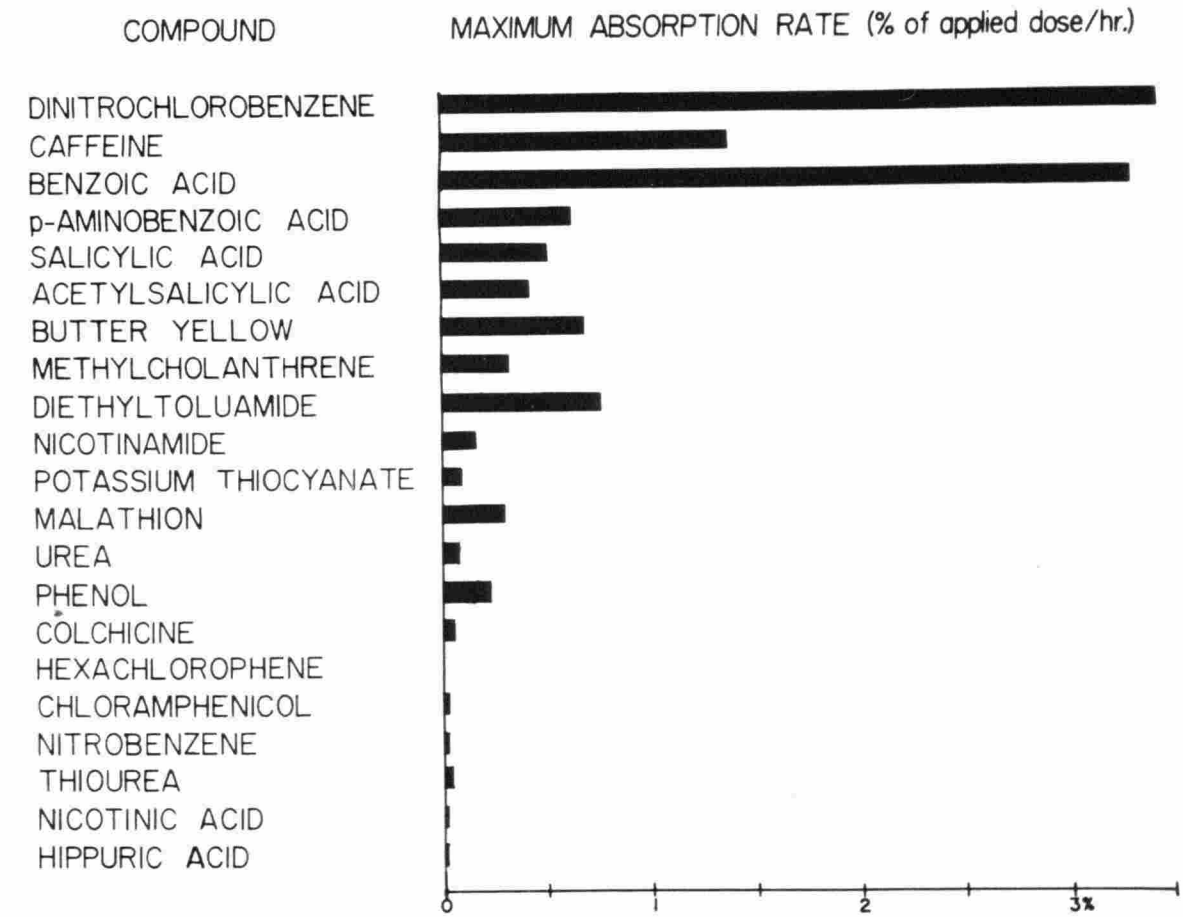

FIG. 2. This represents the maximum absorption rate in % of the applied dose/hour. The chemicals are listed in the same order as in Figure 1. Note the several discrepancies in compounds having a different ranking between highest total absorption and maximum absorption rate.

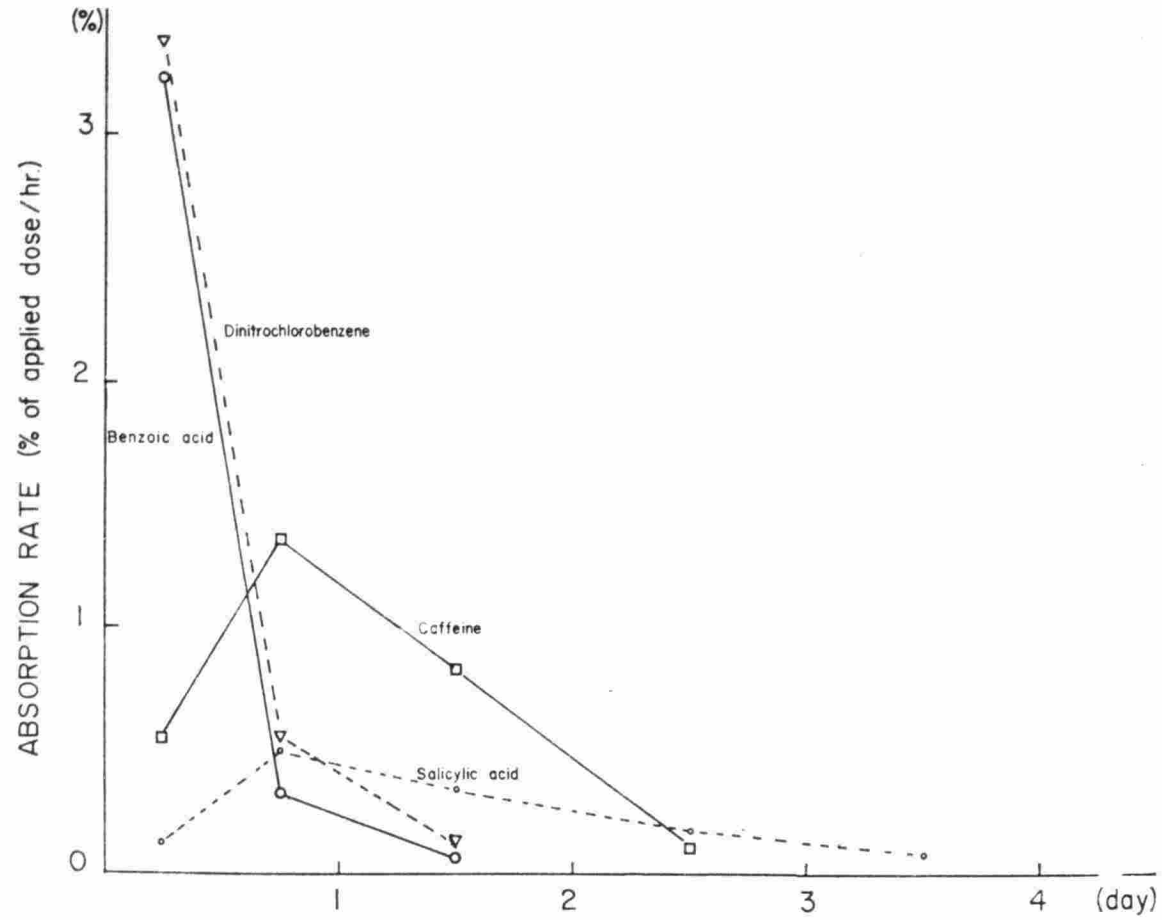

FIG. 3. This figures demonstrates the absorption rate in % of the applied dose/hour for the more extensively absorbed compounds for several days after cutaneous application.

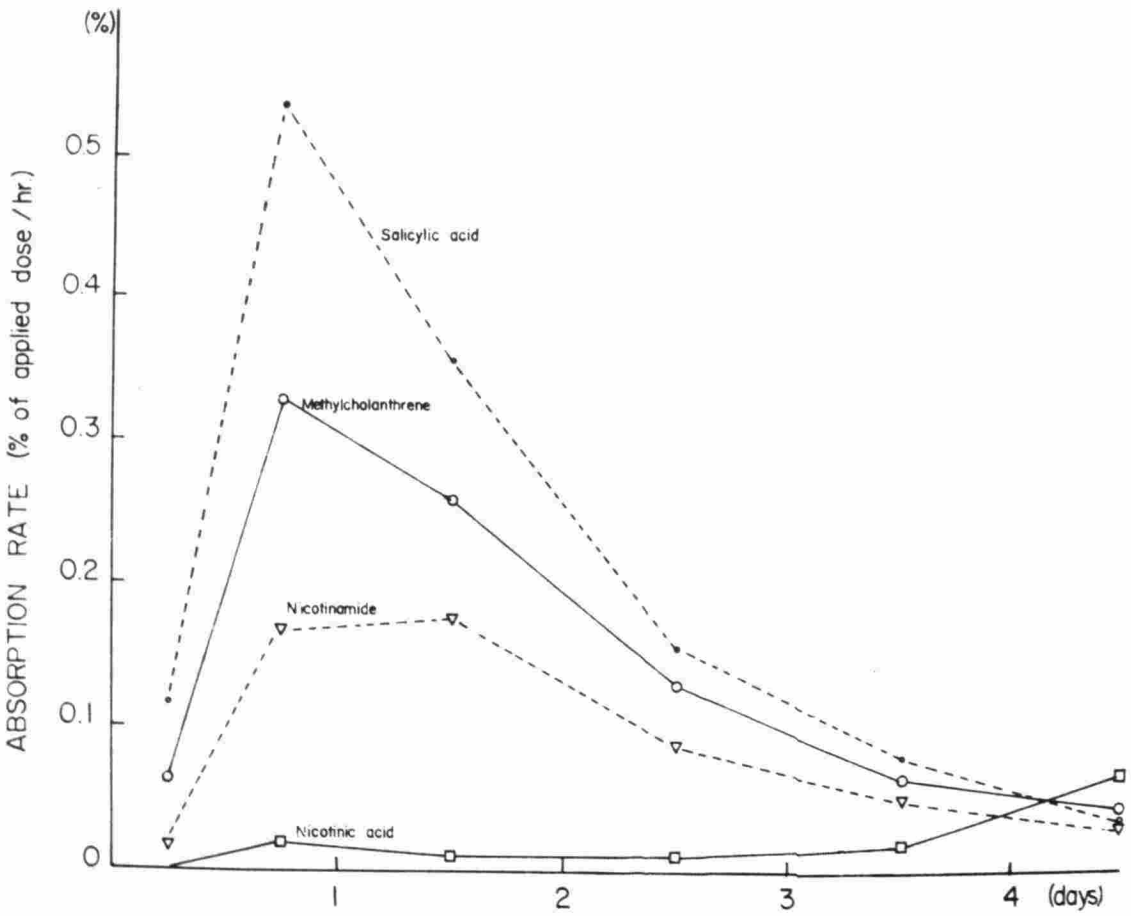

FIG. 4. This figure demonstrates the absorption rate in % of the applied dose/hour for additional compounds having a lower absorption rate than those in Figure 3. Note the scale change from that in Figure 3.

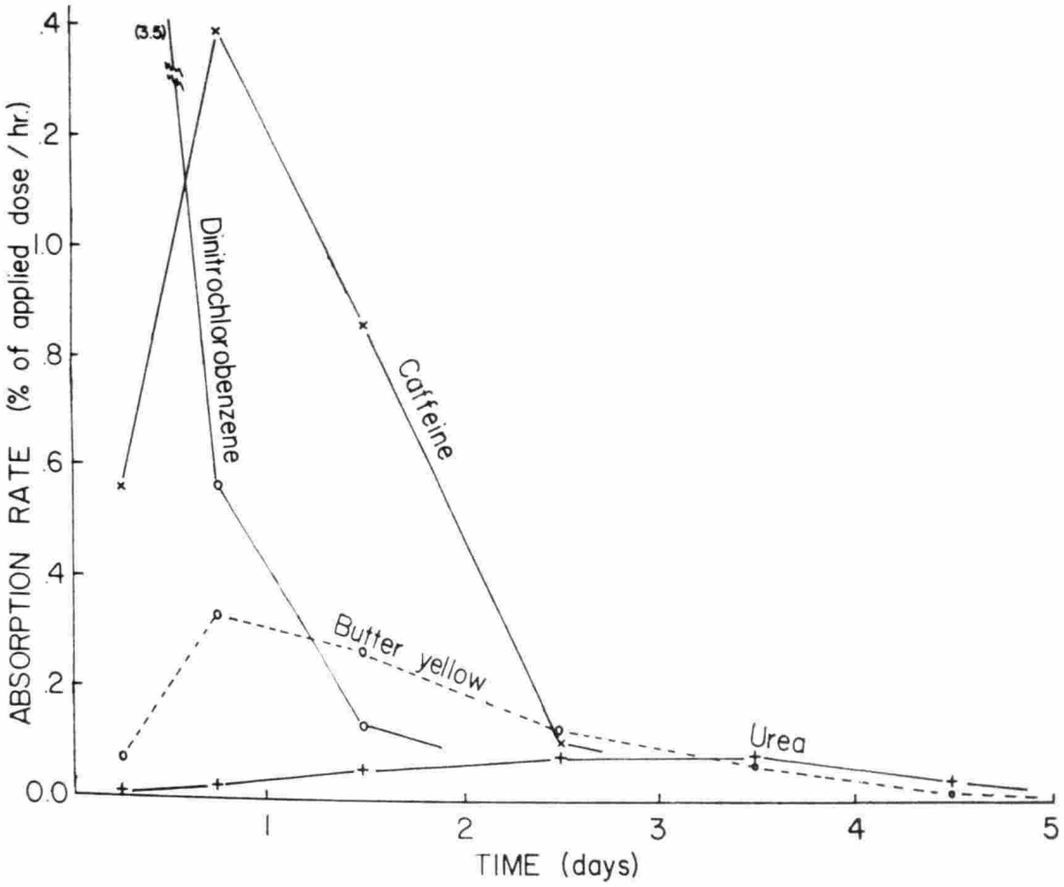

FIG. 5. This is the absorption rate in % of the applied dose/hour for additional compounds. Caffeine and DNCB are repeated for comparison. Note the scale change from Figure 3.

period. The maximum absorption rate observed for each compound is shown in Figure 2 in the same order of compounds as listed in Figure 1. Total absorption depends on both rate of absorption and duration of skin exposure. The discrepancy in order of magnitude in Figures 1 and 2 may be some aspect involving exposure to the compound. Examples relating absorption rate at different times in the experiment are given in Figures 3, 4, and 5. The scales are different in the three figures. Salicylic acid was included in Figures 3 and 4 for comparison.

DISCUSSION

There was a very large difference in penetration of the compounds tested. The range for total absorption was greater than 250 times while differences in maximum absorption rate were greater than 1000 fold. The total penetration of benzoic acid, caffeine, and DNCB were considerably greater than any steroid we studied (1).

The great difference in the total quantities and rates of absorption of the compounds listed is a matter of practical importance. Benzoic acid, salicylic acid and phenol are used in topical dermatological therapy. Butter yellow is an azo dye which is not used itself for dermatologic or cosmetic purposes, but is related to azo dyes which are. Esters of p-aminobenzoic acid are widely used in sunscreen preparations. Diethyltoluamide is the most generally employed insect repellent. Methylcholanthrene and malathion are examples of toxic substances to which there is a danger of environmental exposure. Almost half of the compounds had an absorption greater than 20% of the applied dose.

Two examples are included of closely related compounds showing great differences in penetration. Benzoic acid was absorbed 200 times more than its glycine conjugate—hippuric acid. Nicotinic acid showed minimal penetration while 10% of its amide, nicotinamide, was absorbed. It is possible that similar differences occur in other related chemicals which are applied topically for dermatologic purposes, or are encountered in industrial or other environments. Very few chemicals are completely nontoxic, particularly when parenterally administered.

The total absorption of any chemical will

depend on other factors than the permeability measured in this experiment. No chemical shows complete absorption. Presumably the balance is lost from the epidermal surface. With most compounds the surface loss rate must exceed the absorption rate, for the latter are generally below 50%. We are impressed by the slowness of the penetration of some of these compounds. We assume that these long periods of penetration represent some storage phenomenon; we do not know where this resides or its mechanism. Other factors are the anatomic area, the area of exposure, temperature, humidity, and the presence of disease (3).

Analysis of these data allows a comparison of the total penetration of a compound and the maximum absorption rate in % of the applied dose/hour. In Figure 1 the chemicals are listed in order of greatest total penetration (from the greatest, DNCB, to the least, hippuric acid). Figure 2 gives the data in maximum absorption rate/hour (rather than the total absorption). The same listing arrangement holds in Figure 2. We note that the general ranking from greatest to least is similar but with some exceptions. Caffeine has a higher rank in total penetration than in maximal absorption rate. Benzoic acid, butter yellow and diethyltoluamide represent similar exceptions in that their maximal absorption rates are somewhat out of line with their total absorption. The mechanism for these differences requires explanation. Yet there is generally a good correlation between the maximum penetration rates and the total absorption.

Several of these compounds have been studied previously by others. The published data may not reliably be compared with this *in vivo* human experience as in the literature a variety of test systems were employed. These included *in vitro* and *in vivo* models and several species from mice to men. In fact, the present data represent the first such *in vivo* study in man with a large variety of compounds. Nevertheless, certain comparisons seem in order.

Nicotinic acid was applied to human skin *in vitro* by Cronin and Stoughton (4). They noted no penetration in several specimens and an average of 0.001% of the applied dose in other specimens. Our *in vivo* data also suggest that it is a minimal penetrant.

While there have been extensive studies on esters of salicylic acid, few investigators examined the parent compound. Gemmell and Morrison measured salicylate blood levels in rabbits following applications of 5% salicylic acid in 3 ointment vehicles (5). Burckhard, Wirth and Ganzoni measured urinary salicylates from 10% salicylic acid ointments (6). Stolar and associates recorded blood levels in rabbits after application of a 6% ointment (7). C^{14} labeled salicylic acid was used by Gstirner and Elser to measure residues of ointments containing 5% salicylic acid on rabbit skin (8). Two recent deaths have been reported after whole body application of salicylic acid in the treatment of tinea umbricata (9). Although the experimental methods employed differed from those in this paper, all data suggest the compound is well absorbed.

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