THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 44, No. 5

Printed in U.S.A.

CHEMICAL STRUCTURE AND SKIN PENETRATING CAPACITY OF A SHORT SERIES OF ORGANIC PHOSPHATES AND PHOSPHORIC ACID*

FRANCIS N. MARZULLI, PH.D.,** JOHN F. CALLAHAN, B.S.,*** AND D. W. C. BROWN, (M.A.)**

A powerful tool for investigating mechanisms of skin penetration is provided by employing systematic, albeit minor, modifications in molecular structure of type compounds. By applying this technic, it is possible to identify structural, chemical and physical properties of substances associated with the capacity to penetrate skin.

Valette and Cavier (1, 2, 3, 4) employed this method in studying vehicles which facilitate the penetration of eserine through skin. Effectiveness, in their studies, was measured by contractibility of rat striated muscle, a pharmacologic endpoint.

In the present work, quantitative results were obtained with a human skin model which appears to be capable of predicting *in vivo* skin penetration quite accurately. Steady state rates of penetration of P^{32}- and C^{14}-labeled compounds were measured by using sheets of anterior forearm stratum corneum conjunctum (S.C.C.) in diffusion cells.

The technic consisted of mounting s.c.c. discs in the cells and bathing the tissues' under-surface with a constant flow of physiological saline. The radioactive labeled material was applied to the upper surface of the membrane and collected by the effluent as it penetrated. Chemical analysis of the effluents' radioactivity determined the amount of penetration. Analytical accuracy of the technic was found to be at least 85% efficient. More complete details of the technic have been described previously (5).

A limited proof of the validity of the test method for use with the present series of compounds is indicated by studies with tri-n-butyl phosphate, a representative member. In these studies it was found that the average maximum steady state rate of penetration of tri-n-butyl phosphate through the anterior forearm skin of three subjects (6) was essentially the same as through isolated full thickness skin in a diffusion chamber (0.10 vs 0.05 $\mu g/cm^2/min$). The limiting feature of this analogy, however, is the fact that it compares only one compound; and the measurements were made by a method which can overestimate actual penetration rate and is not always a reliable index of systemic absorption. In spite of these numerous qualifying factors it is felt that the information developed is, to date, the only validating work; and therefore one can attempt to consider the results in this theoretical concept.

In the present work the skin penetrant capacities of related, undissociated phosphorus-containing compounds were investigated. These are trimethyl (TMP), triethyl (TEP), tri-*n*-propyl (TNPP), tri-isopropyl (TIPP), tri-*n*-butyl (TNBP), trio-*o*-cresyl (TOCP) and diethyl-*o*-cresyl (DEOCP) phosphate. For purposes of comparison with an ionized form, phosphoric acid was also studied.

RESULTS

A summary of results is given in Table I in which chemical and physical properties of the test substances are listed together with their quantitative skin penetration rates.

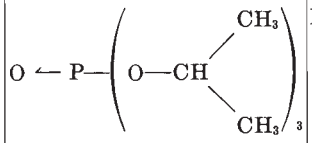
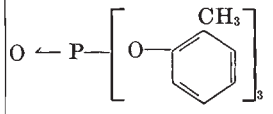
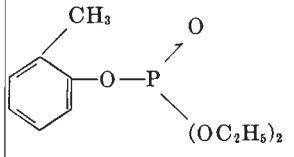
Statistical analysis of data shows significant differences in 27 cases in which skin penetration rates for different compounds were paired. In one case, significance is not observed. Four of the twenty-seven significantly different pairs are not significant when the gram is the unit of comparison rather than the mole. Thus, the probability of seeing significant differences among skin penetration rates is increased by changing the form of the data from $\mu g/cm^2/min$ to $\mu moles/cm^2/min \times 10^2$.

A decrease in penetrant capacity is observed among the trialkyl phosphates as the carbon chain length is increased from TMP to TNPP

This work was initiated when the senior author was at Edgewood Arsenal, Maryland.
Received for publication June 25, 1964.
* From the Dermal Toxicity Branch, DP, Bureau of Scientific Research,** Food and Drug Administration, Washington, D. C. and the Field Toxicology Branch, Toxicology Division, Chemical Research & Development Laboratories,*** Edgewood Arsenal, Maryland.

TABLE I

Penetration of related organic phosphates and phosphoric acid through isolated human stratum corneum conjunctum

Compound	Formula	Label	Mol. Wt.	B.Pt.-760 mm° C	Density d_4^{25}	Solubility Benz/water pts/100		Part. Coef. Benz/water Kd^{25}	Maximum Steady State Penetration Rate	
									μg/cm²/min	μmoles/cm²/min × 10^2
A Trimethyl phosphate (9)	$O \leftarrow P-(OCH_3)_3$	P^{32}	140	197 (1)	1.2052 (1)	misc.	>400 (6)	0.5 (8)	1.47 (1.12–1.81)	1.047 (0.801–1.290)
B Triethyl phosphate (9)	$O \leftarrow P-(OC_2H_5)_3$	P^{32}	182	210 (1)	1.0695 (1)	misc.	>100 (6)	13 (8)	1.12 (0.75–1.50)	0.623 (0.437–0.808)
C Tri-n-propyl phosphate (9)	$O \leftarrow P-(OCH_2CH_2CH_3)_3$	P^{32}	224	252 (1)	1.0023 (1)	misc.	0.6 (6)	187 (8)	0.65 (0.44–0.85)	0.288 (0.194–0.382)
C′ Tri-isopropyl phosphate (9)		P^{32}	224	218 (1)	0.9867 (1)	misc.	1.2 (6)	84 (8)	0.78 (0.48–1.08)	0.350 (0.217–0.483)
D Tri-n-butyl phosphate (9)	$O \leftarrow P-(OC_4H_9)_3$	P^{32}	266	289 (1)	0.9727 (1)	misc.	0.04 (7)	229 (7)	0.18 (0.05–0.31)	0.067 (0.019–0.115)
E Tri-o-cresyl phosphate (9)		P^{32}	368	410 (2)	1.1718 (5)	misc.	0.005 (7)	5800 (7)	0.003 (0.001–0.004)	0.0007 (0.0004–0.0011)
F Di-ethyl-o-cresyl phos. (10)		C^{14}	244	229 (3)	1.1168 (12)	misc.	0.4 (7)	151 (7)	0.35 (0.23–0.40)	0.128 (0.092–0.165)
G Phosphoric Acid, 85% (11)	$O \leftarrow P-(OH)_3$	P^{32}	98	158 (4)	—	0.0000 (7)	misc.	<0.006 (7)	—	—
G′ Phosphoric Acid, 8.5%	—	P^{32}	—	100 (4)	—	0.0008 (7)	misc.	<0.006 (7)	0.003 (0.002–0.005)	0.003 (0.002–0.005)

(1) Survey of Organic Phosphorus Compounds, J. R. Costello & J. C. Price, U. of Notre Dame Summary Report, 8/15/59.
(2) Chem. Rubber Handbook, 44th Ed., 1963.
(3) Determined by L. Ford, FDA.
(4) Merck Index, 1960.
(5) Evans, David, Philip & Jones, Wm. Jacob, "The Viscosity of Phosphone Esters", Chem. Society (London) Journal 985 (1932).
(6) Determined by cloud pt. by J. J. Callahan, Edgewood Arsenal, Maryland.
(7) Determined by D. Brown, FDA & W. C. Alford, NIH.
(8) Determined by J. F. Callahan, Edgewood Arsenal, Maryland.
(9) Prepared by New England Nuclear.
(10) Prepared by B. Kagan & L. Ford, FDA.
(11) Obtained from Oak Ridge, Tennessee.
(12) Determined by L. Dow, FDA.

Differences in penetration rate which are significant at the 5% level by the "t" test (μmoles/cm^2/min.). Calculated by J. Van Dyke, FDA. A-B, A-C, A-C′, A-D, A-E, A-F, A-G′, B-C, B-C′, B-D, B-E, B-F, B-G′, C-D, C-E, C-F, C-G′, C′-D, C′-E, C′-F, C′-G′, D-E, D-F, D-G′, E-F, E-G′, F-G′.

Not significantly different C-C^1.

to TNBP. No significant differences in skin penetration are observed between tri-n-propyl and tri-isopropyl phosphates. The tri-aryl phosphate (TOCP) is like phosphoric acid in its relative inability to penetrate our skin model. Diethyl-o-cresyl phosphate (DEOC), which was synthesized to study a combination aliphatic-aromatic phosphate, appears to be intermediate in skin penetrant capacity, with a mean steady state rate between that of the aliphatic TEP and the aromatic TOCP.

When all compounds in this series, both dissociated and undissociated, are evaluated, the absolute solubility in benzene and in water shows a good correlation with skin penetration. This is also true if the benzene-water distribution ratio at 25° C. (Kd^{25}) is used. Here, those substances whose benzene/water partition ratio is closest to 1 are associated with the highest penetration rates.

If we limit our comparison to those substances which do not ionize, we observe a good correlation between molecular weight and skin penetration rate (Figure 1). In this series of compounds, the lower boiling materials appear to be better skin penetrants than those with higher boiling points.

DISCUSSION

The main advantage in the use of an *in vitro* rather than *in vivo* technic (for measuring skin penetration), such as employed with our skin model, is simplicity. The advantage of using human rather than animal tissue for extrap-

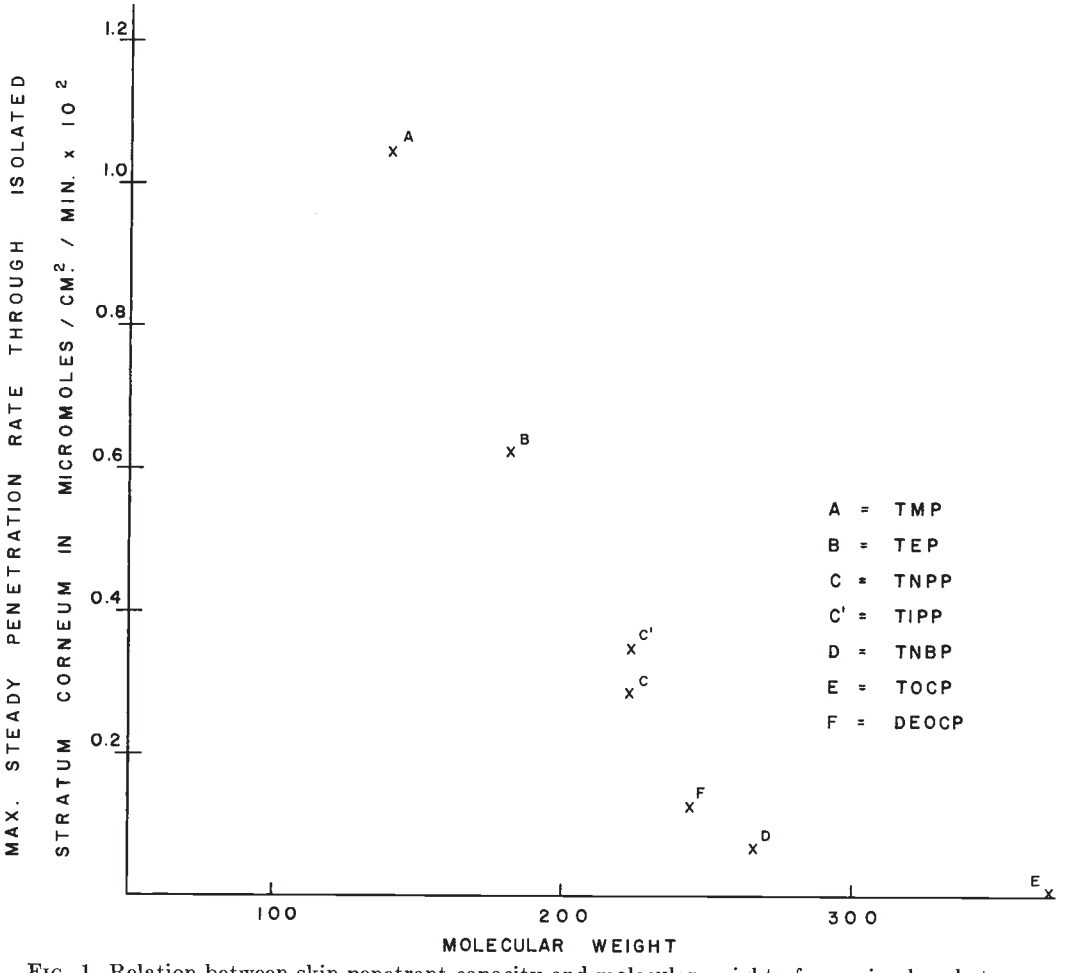


FIG. 1. Relation between skin penetrant capacity and molecular weight of organic phosphate compounds.

olation to man is obvious. A major objection to this type of skin penetration study is that dead skin is employed, hence results may not be directly applicable to living man. Since the dead tissue used in our model (stratum corneum conjunctum) is not alive when performing its barrier function on the skin of man *in vivo*, this objection would appear unwarranted. If, as appears to be the case, this tissue structure provides the major rate-limiting factor in the transport of substances through skin, it matters little that *more* rapid clearance is possible through underlying tissues. Since no one has yet demonstrated an active transport mechanism in the skin of man (or mammals) similar to that of frogs, capable of acting against a concentration gradient, there is probably small likelihood that an additional barrier system such as this functions in the skin of man.

The barrier function of skin enzymes which may detoxify and otherwise alter skin penetration is still unclear. Experimental evidence for such a system has, however, been reported by Fredriksson (7).

One reason for obtaining precise skin penetration data, such as obtained with isolated tissues, is the possibility that we can more quickly arrive at adequate equations for predicting absolute rates of transport of various types of substances through human skin *in vivo*. Since skin is unique in its structure, mechanisms which explain penetration of substances through other organ systems may not be directly applicable. Different rules may be required to explain how electrolytes and non-electrolytes penetrate. Thus a comprehensive formula for skin penetration may require that the degree of dissociation of the test material be considered. At present, it is felt that in general, neutral molecules pass through skin readily, while ionized forms traverse with great difficulty, regardless of size. It is not surprising, then, that in our series the ionized phosphate (phosphoric acid) is not a good skin penetrant. It is not yet clear, however, at what absolute rates different varieties of non-ionized substances with similar molecular weight and similar oil/water solubility characteristics penetrate skin. It is apparent that molecular size, structure, solubility and electrochemical properties are all important.

One of the difficulties in isolating the effects of a particular physical characteristic on skin penetration in a homologous series is the potential for masking by one of two distinctive, yet parallel properties. A rise in density and in boiling point in a homologous series, for example, both accompany a rise in molecular weight. A correlation which is seen between the densities of a series of compounds and their skin penetration rates could, therefore, be coincidental.

Benzene/Water Partition Coefficient

One of the earliest empirical rules relating biologic activity to a physical property is the well known Overton-Meyer hypothesis, *i.e.*, that narcotic effect is related to the lipoid/aqueous distribution ratio. That this ratio also bears a good relation to skin penetration is shown by results with the members of our organic phosphate series. Treherne (8) reported a high correlation between rabbit skin penetration (*in vitro*) and the aqueous/lipoid distribution ratio of a series of unrelated non-electrolyte substances. Similarly, Clendenning and Stoughton (9) in a study of closely related boronic acid derivatives showed that penetration of human skin (*in vitro*) was related to the partition coefficient.

Solubility

One of the most useful and predictable properties of an organic compound from the standpoint of characterization is its solubility behavior toward different solvents. In a homologous series, solubility decreases by a definite amount for each additional CH_2 link. Addition of OH groups increases water solubility. Certain biologic changes would be expected to parallel changes in solubility characteristics. Among the better known mechanisms in which solubility is important to the body is that of detoxication. Here, for example, toxic substances may be converted to more water-soluble derivatives. By reducing renal reabsorption, such substances are eliminated faster (10). In the present studies, the absolute solubility in benzene and in water provides a good clue as to the skin penetrant capacity of each substance. The best penetrant (TMP) is highly soluble in both benzene and in water. The poorest skin penetrants show quite limited solubility in either benzene (phosphoric acid) or in water (TOCP). Compounds with intermediate solubility properties are intermediate in

skin penetration. In this series, both aqueous and lipid solubility appear to be required to be a good penetrant.

Molecular Weight

The well known fact that large molecules are less capable of traversing membranes of any type provides a basis for considering molecular size as a factor in skin penetration. A sieve-like structure in membranes has been postulated to account for this type of restriction. In the present studies, the data plotted in Figure 1 show a rather precise relation between molecular size and skin penetration, when limited to the undissociated phosphates. Molecular shape may also determine the rate of transport of substances through skin; however, this was not demonstrated in the single instance where this factor might be assessed (compare the straight-chain structure tri-*n*-propyl phosphate with the branched-chain tri-isopropyl phosphate).

Boiling Point

The boiling point of a liquid shows a definite and well known relation to structure. In a homologous series, the boiling point rises with increasing carbon-chain length and corresponding increase in molecular weight.* From these facts alone, one would predict that the lower boiling members of our series would be the better skin penetrants. And, indeed, there is a good correlation between these two factors. On the basis of its lower boiling point as well as its greater solubility, TIPP would be expected to be a better skin penetrant than its isomer (TNPP). The actual results obtained show a trend in this direction but the differences are not significant. From a historical point of view, volatile substances, namely gases, were among the first substances known to penetrate skin (11).

SUMMARY

1. The skin penetrant capacities of a short series of organic phosphates and phosphoric acid were measured by using a skin model in which isolated anterior forearm stratum corneum conjunctum was employed.

2. Average maximum steady state rates of penetration (with 19/20 limits) in μmoles/cm^2/min x 10^2 were as follows:

Trimethyl phosphate—1.047 (0.801–1.290)
Triethyl phosphate—0.623 (0.437–0.808)
Tri-*n*-propyl phosphate—0.288 (0.194–0.382)
Tri-isopropyl phosphate—0.350 (0.217–0.483)
Tri-*n*-butyl phosphate—0.067 (0.019–0.115)
Tri-*o*-cresyl phosphate—0.0007 (0.0004–0.0011)
Di-ethyl-*o*-cresyl phosphate—0.128 (0.092–0.165)
Phosphoric acid—0.003 (0.002–0.005)

3. For this group of substances, absolute solubility in both benzene and in water, and benzene/water distribution are correlated with skin penetrant capacity.

4. Both molecular weight and volatility of the undissociated organic phosphates are related to their skin penetrant capacities.

* Replacement of the H of a hydroxyl group by an alkyl group, on the other hand, lowers the boiling point while increasing the molecular weight, *e.g.*, compare water and ethyl alcohol.

REFERENCES

1. Vallette, G. and Cavier, R.: Sur un test physiologique utilisable pour l'etude de la vitesse d'absorption d'un medicament. Comptes Rendus Soc. Biol., Paris, *144:* 825, 1950.
2. Vallette, G. and Cavier, R.: Sur un test physiologique applicable a l'etude de la vitesse d'absorption d'un medicament. J. Physiol. (Paris), *43:* 41, 1951.
3. Vallette, G. and Cavier, R.: Absorption percutanee et constituion chemique. Arch. Int. Pharmacodyn., *97:* 232, 1954.
4. Vallette, G., Cavier, R. and Savel, J.: Les Facteurs physiques de l'absorption cutanee des liquides organiques. Arch. Int. Pharmacodyn., *97:* 241, 1954.
5. Marzulli, F. N.: Barriers to skin penetration. J. Invest. Derm., *39:* 387, 1962.
6. Tregear, R. T., C.D.E.E., Porton, Wiltshire, England, personal communication.
7. Fredriksson, T.: Studies on the percutaneous absorption of parathion and paraoxon. J. Invest. Derm., *38:* 233, 1962.
8. Treherne, J. E.: The permeability of skin to some non-electrolytes. J. Physiol. (London), *133:* 171, 1956.
9. Clendenning, W. E. and Stoughton, R. B.: Importance of the aqueous/lipid partition coefficient for percutaneous absorption of weak electrolytes. J. Invest. Derm., *39:* 47, 1962.
10. Brodie, B. B. and Hogben, C. A. M.: Some physico-chemical factors in drug action. J. Pharm. Pharmacol., *9:* 345, 1957.
11. Barr, M.: Percutaneous absorption. J. Pharm. Sci., *51:* 395, 1962.