COMPARATIVE STUDIES ON PERCUTANEOUS ABSORPTION

By D. H. O. Gemmell and J. C. Morrison

From the School of Pharmacy, The Royal College of Science and Technology, Glasgow

Received May 14, 1958

The percutaneous absorption of salicylic acid, sulphanilamide, copper acetyl-acetonate, and copper sulphate through the intact skin of rabbits and the efficiency of lard, emulsifying ointment B.P., and water in the form of a five per cent carboxymethyl cellulose gel, as carriers, is compared. Blood levels were accepted as a measure of absorption. The physico-chemical properties of the drugs seemed to dictate the amount absorbed; the influence of the base was also significant, although less so. Best absorbed was salicylic acid, next sulphanilamide, then copper acetyl-acetonate, and finally copper sulphate, although the differences were slight between the last two; lard was the best base, then emulsifying ointment, and finally water.

The *in vivo* methods used to study percutaneous absorption include blood, urine, faeces and tissue analyses and pharmacological, histological and clinical methods[1]. We have used the blood levels attained after the topical application of sulphanilamide to compare the efficiency of vehicles or bases as "carriers"[2]. The present study examines the amount of percutaneous absorption of four different drugs, namely, salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate, from three vehicles or bases, namely, lard, Emulsifying Ointment B.P. and water, as a 5 per cent carboxymethylcellulose gel.

After their cutaneous application, the detection and estimation of the drugs in the bloodstream of the animal permitted a comparative study of the efficiencies of the vehicles and bases and the effect on absorption of the properties of the drugs incorporated in these bases. The series of experiments undertaken were designed to provide sufficient results to make a complete statistical examination.

Experimental

Choice of Drugs

The drugs were selected to demonstrate a wide range of pharmacological and chemical properties but the choice was limited to those readily and accurately determined in blood. They were: salicylic acid, a lipid soluble organic acid, soluble in alcohol, ether, glycerol and chloroform, and possessing keratolytic and irritant properties; sulphanilamide, a neutral organic compound, soluble in water, ethanol, acetone and glycerol; copper acetyl-acetonate, a non-ionic compound of copper chelated with acetyl-acetone, insoluble in water, but soluble in alcohol and chloroform, and copper sulphate, an ionic compound, readily soluble in water and glycerol but insoluble in alcohol.

Choice of Vehicles

Previously reported experiments[2] with sulphanilamide had shown lard to be the most efficient of the vehicles and bases tested and water, as a

5 per cent carboxymethylcellulose gel, to be the least efficient. Emulsifying Ointment B.P. gave an efficiency value intermediate between these two[2]. It was decided to incorporate the four drugs in these bases to determine whether the same order of efficiency of the vehicles and bases held for drugs other than sulphanilamide.

Concentration of incorporated drug. The concentration of drug first used in the vehicles and bases was 10 per cent weight-in-weight. But with salicylic acid the concentration was lowered to 5 per cent to reduce the local irritant and keratolytic effect. This decrease in concentration would be unlikely to invalidate comparative blood level studies since others had found that an increase to over 5 per cent in the concentration of the incorporated drug did not lead to an increase in absorption[3].

Design of test and experimental details. These have been described in a previous publication[2] where a suitable test was designed for the comparison of vehicles and bases as "carriers" of drugs through the intact skin of rabbits.

Estimation of Drugs in Blood Samples

Salicylic acid. A modification of Smith and Talbot's colorimetric method was used[4]. 0·2 ml. of blood was added to 3·2 ml. distilled water followed by 0·6 ml. of a 25 per cent aqueous solution of trichloroacetic acid. To 2 ml. of the filtrate, 1 ml. 1·5N sodium hydroxide was added. Folin-Ciocalteu's reagent, 0·5 ml., diluted one to four with distilled water, was then added and the volume adjusted to 5 ml. with 1·5N sodium hydroxide. After standing, the solutions were filtered and extinctions measured against a reagent blank at a wavelength of 690 mμ. To correct for non-specific plasma phenols which are included in this estimation, the average reading of 36 normal blood samples treated as above was subtracted from the observed values before referring to a calibration curve, constructed by similarly treating known dilutions of sodium salicylate in distilled water. The concentration of reductable material in blood estimated by this method gave a value of 10·41 mg./100 ml.

Sulphanilamide. Sulphanilamide in blood was estimated by King's micro-modification of the method of Bratton and Marshall[5].

Copper. Copper, in ammoniacal solution, was estimated colorimetrically with sodium diethyl dithiocarbamate which is capable of detecting one part of copper in fifty million parts of solution. The colour produced is stable for several hours[6].

0·2 ml. blood was added to 3·2 ml. distilled water followed by 0·6 ml. of a 25 per cent aqueous solution of trichloroacetic acid. To 2 ml. of the filtrate was added 0·5 ml. of a 10 per cent solution of ammonia. Three drops of a 0·1 per cent solution of sodium diethyl dithiocarbamate were then added and the final volume adjusted to 5 ml. with distilled water. The extinctions of the solutions were measured against a reagent blank at a wavelength of 455 mμ. Estimated by this method, the concentration of copper present in normal circulating rabbit blood, taking the average of 24 blood samples, gave a value of 0·18 mg./100 ml. Copper solutions of known concentration were added to freshly drawn rabbit blood and the

procedure repeated. The estimation of copper was accurate to within 1 per cent so that, under the conditions of the assay, any complexes formed by the metal and the blood proteins appear to have broken down, the copper remaining in solution as the stable trichloroacetate.

RESULTS

The results are summarised and shown in Tables I, II, III, and IV; the statistical analysis is given in Table V.

TABLE I

COMPARISON OF PERCUTANEOUS ABSORPTION IN THE RABBIT OF SALICYLIC ACID, SULPHANILAMIDE, COPPER ACETYL-ACETONATE AND COPPER SULPHATE, FROM LARD, EMULSIFYING OINTMENT B.P. AND A 5 PER CENT CARBOXYMETHYLCELLULOSE GEL

Drug	Base	Mean blood level of drug observed in 6 individual rabbits over 8 hours, mg./100 ml.						Mean blood level of drug observed in 6 rabbits over 8 hours, mg./100 ml.
		Rabbit Number						
		1	2	3	4	5	6	
Salicylic acid	Lard	1·09	1·13	1·24	1·21	1·13	1·17	1·16
	Emulsifying Ointment B.P.	0·86	0·84	1·01	0·99	1·08	1·09	0·98
	Water as a 5 per cent carboxymethyl cellulose gel	0·90	0·85	0·81	1·03	0·92	0·74	0·88
Sulphanilamide	Lard	0·88	1·03	0·91	1·02	0·82	0·75	0·89
	Emulsifying Ointment B.P.	0·48	0·56	0·53	0·39	0·48	0·35	0·47
	Water as a 5 per cent carboxymethyl cellulose gel	0·28	0·28	0·29	0·48	0·44	0·37	0·36
Copper acetyl-acetonate	Lard	0·25	0·25	0·28	0·26	0·24	0·26	0·26
	Emulsifying Ointment B.P.	0·23	0·23	0·24	0·21	0·22	0·23	0·23
	Water as a 5 per cent carboxymethyl cellose gel	0·19	0·19	0·19	0·18	0·17	0·19	0·19
Copper sulphate	Lard	0·21	0·21	0·22	0·19	0·24	0·24	0·22
	Emulsifying Ointment B.P.	0·20	0·21	0·21	0·20	0·20	0·23	0·21
	Water as a 5 per cent carboxymethyl cellulose gel	0·19	0·17	0·17	0·18	0·18	0·20	0·18

Table I shows the mean blood level obtained for the individual rabbits throughout the test. These figures are calculated by summing all the 16 observed half-hourly levels for each rabbit and dividing by the number of blood samples taken, i.e., 16. They provide a useful and practical index of efficiency for a particular vehicle or base.

The average response is found by summing the above totals over all six rabbits and dividing by the new total number of observations i.e., 96. The analysis was worked on totals rather than means since the former require fewer arithmetical operations and are less liable to error. These totals are given in Table II.

Table III shows the total amount observed in six rabbits for one drug from a particular vehicle or base and the mean rabbit response to the drug in that vehicle or base under the conditions of test.

Table IV is a table of comparative efficiencies and shows the order of efficiency of the three vehicles or bases for each drug tested. For each drug, lard is the most efficient vehicle or base; water, as a 5 per cent carboxymethylcellulose gel, is the least efficient. The extent of absorption

TABLE II

COMPARISON OF PERCUTANEOUS ABSORPTION IN THE RABBIT OF SALICYLIC ACID, SULPHANILAMIDE, COPPER ACETYL-ACETONATE AND COPPER SULPHATE, FROM LARD, EMULSIFYING OINTMENT B.P. AND WATER, AS A 5 PER CENT CARBOXYMETHYLCELLULOSE GEL

Drug	Base	Total amount observed in the blood of 6 individual rabbits over 8 hours (each value is the sum of 16 samples), mg./100 ml. Rabbit Number 1	2	3	4	5	6	Total amount of drug observed in 6 rabbits over 8 hours, mg./100 ml.
Salicylic acid	Lard	17·56	18·04	19·80	19·42	18·15	18·72	111·69
	Emulsifying Ointment B.P.	13·71	13·47	16·18	15·86	17·33	17·27	93·82
	Water, as a 5 per cent carboxymethyl cellulose gel	14·45	13·65	13·02	16·42	14·78	11·78	84·10
Sulphanilamide	Lard	14·14	16·05	14·54	16·24	13·03	11·92	85·92
	Emulsifying Ointment B.P.	7·62	9·08	8·45	6·27	7·71	5·57	44·70
	Water, as a 5 per cent carboxymethyl cellulose gel	4·46	4·47	4·64	7·67	7·19	5·93	34·36
Copper acetyl-acetonate	Lard	3·96	4·05	4·54	4·19	3·85	4·24	24·83
	Emulsifying Ointment B.P.	3·71	3·67	3·78	3·42	3·48	3·74	21·80
	Water, as a 5 per cent carboxymethyl cellulose gel	3·03	2·97	2·99	2·94	2·74	3·00	17·67
Copper sulphate	Lard	3·40	3·37	3·47	3·18	3·78	3·83	21·03
	Emulsifying Ointment B.P.	3·27	3·41	3·35	3·21	3·17	3·65	20·06
	Water, as a 5 per cent carboxymethyl cellulose gel	2·99	2·75	2·77	2·94	2·89	3·21	17·55

of the incorporated drugs can also be seen. Salicylic acid was absorbed more readily than sulphanilamide while the acetyl-acetonate and the sulphate of copper were poorly absorbed by comparison.

Table V shows the statistical analysis of variance on the results. The wide variation in the level of reaction to the different drugs and the wide

TABLE III

COMPARISON OF PERCUTANEOUS ABSORPTION OF THE FOUR DRUGS IN THE RABBIT FROM THREE VEHICLES OR BASES

	Lard		Emulsifying Ointment B.P.		Water, as a 5 per cent gel	
	Total amount observed in 6 rabbits, mg.	Mean individual rabbit response, mg.	Total amount observed in 6 rabbits, mg.	Mean individual rabbit response, mg.	Total amount observed in 6 rabbits, mg.	Mean individual rabbit response. mg.
Salicylic acid	111·69	18·62	93·82	15·64	84·10	14·02
Sulphanilamide	85·92	14·32	44·70	7·45	34·36	5·73
Copper acetyl-acetonate	24·83	4·14	21·80	3·63	17·67	2·95
Copper sulphate	21·03	3·53	20·06	3·34	17·55	2·93

variation in the variances makes the result of an analysis of variance rather more difficult to interpret than in the orthodox case.

Because of the death of two of the test animals it was impossible to replicate the determinations for sulphanilamide. Accordingly, these results were excluded from the analysis of variance which thus became a two factor analysis with replication. The difference between drugs was highly significant although the difference between the two compounds of copper tested by the *t*-test was not significant. This implied that there was a very large difference between the vehicles and bases containing salicylic acid and those containing the two compounds of copper.

TABLE IV

TABLE OF COMPARATIVE EFFICIENCIES

THE BLOOD LEVEL FOR COPPER SULPHATE IN WATER, AS A 5 PER CENT CARBOXYMETHYLCELLULOSE GEL, TAKEN AS UNITY

	Lard	Emulsifying Ointment B.P.	Water, as a 5 per cent carboxy-methylcellulose gel
Salicylic acid	6·32	5·32	4·75
Sulphanilamide ..	4·85	2·54	1·95
Copper acetyl-acetonate	1·42	1·23	1·01
Copper sulphate ..	1·19	1·14	1·00

The drug-base interaction was significant at a level between $P = 0{\cdot}01$ and $P = 0{\cdot}05$. Examination showed that this was almost entirely due to the unexpectedly high efficiency of the salicylic acid-lard interaction. The apparently large differences between the bases was found to be due almost entirely to this interaction and when allowance was made for this, the differences between the bases were not significant.

TABLE V

ANALYSIS OF VARIANCE

Source of Variance	Degrees of Freedom	Sum of Squares	Mean Square	F	Remarks
Between Drugs ..	2	21351	11576	116	F = 61·3 for P = 0·001
Between Vehicles or bases	2	489·8	244·9	2·72	F = 6·9 for P = 0·05
Drug x Vehicle or base interaction ..	4	359·6	89·89	4·42	F = 3·6 for P = 0·05
Residual	9	182·8	20·31		F = 6·4 for P = 0·01

Incorporation of the sulphanilamide experiments modified these conclusions as follows:

(1) There were significant differences between salicylic acid, sulphanilamide, and the two compounds of copper, although these did not differ significantly from each other.

(2) Lard is particularly effective as a "carrier" for both salicylic acid and sulphanilamide.

(3) The experiments have not been sufficiently sensitive to give differences between vehicles or bases large enough to produce significant results in the analysis.

Nevertheless, when account is taken of the manner in which differences between the bases occur (lard was always shown to be more efficient than Emulsifying Ointment B.P. which was always more efficient than water, as a five per cent carboxymethylcellulose gel) it is almost certain that more extensive and refined experiments would show such differences between bases to be significant.

Discussion

Salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate, when applied in lard, Emulsifying Ointment B.P. and water, as a 5 per cent carboxymethylcellulose gel, to the intact skin of rabbits enter the circulation in measurable quantities. The order of efficiency of vehicles or bases as "carriers" remained as found previously[2]; lard proved to be the most efficient and water, as a 5 per cent carboxymethylcellulose

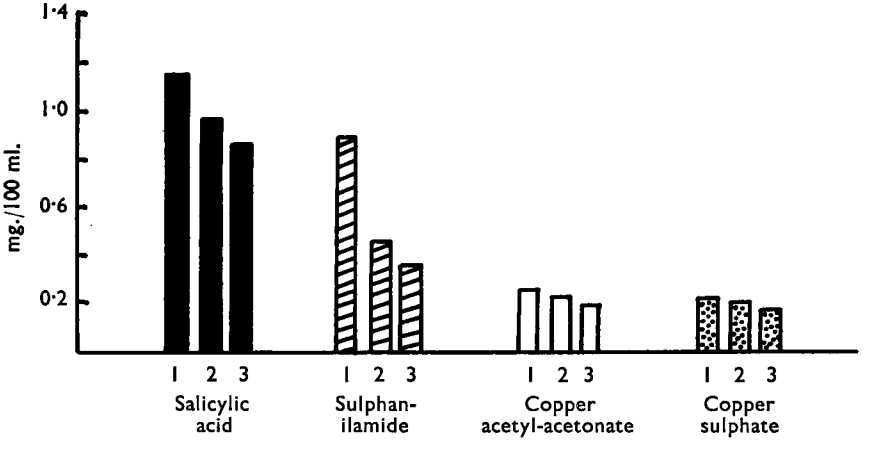

FIG. 1. The mean blood levels of salicylic acid, sulphanilamide, copper acetyl-acetonate and copper sulphate seen in rabbits after their application in lard (1), Emulsifying Ointment B.P. (2) and water (3) as a 5 per cent carboxymethylcellulose gel.

gel, to be the least efficient. It seems that the physico-chemical nature of the applied drugs is of the greatest importance in dictating absorption through the skin. This is clearly illustrated in Figure 1. That these properties outweigh the influence of the vehicle is well shown by a consideration of the data shown in Figure 2. Indeed, it is surprising that the differences in absorption of each drug from water, emulsifying ointment and lard are so small.

The differences in the amounts of drugs absorbed from the three bases may be explained in terms of their local actions and lipid solubilities. Whether other pharmacological properties play a part in the absorption of drugs is a question for further enquiry. Others have suggested that the known irritant and keratolytic action of salicylic acid[7,8] may influence absorption. Certainly in our experiments localised vasodilatation was observed after removal of the salicylic acid preparations

from the backs of the animals. Sulphanilamide is neutral and appears to exert neither an irritant nor an astringent effect when applied topically and probably produces no reactions which would be expected to increase or decrease percutaneous absorption. In contrast, copper is astringent and is used locally for its precipitant action on proteins. Whether this action influences the absorption of copper is still unknown. The slightly higher concentrations of copper acetyl-acetonate may be due to the non-ionic character of the copper which is chelated with acetyl-acetone molecules increasing its lipid solubility in contrast to the lipid insolubility of the

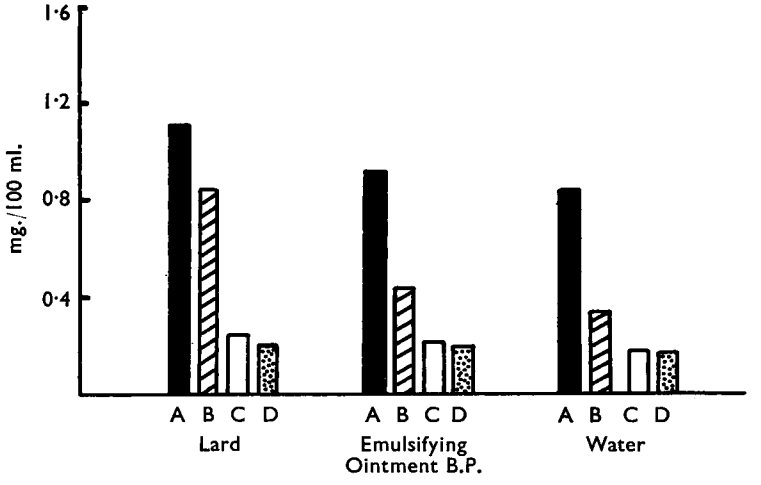

FIG. 2. The mean blood levels of salicylic acid (A), sulphanilamide (B), copper acetyl-acetonate (C) and copper sulphate (D) seen in rabbits after their application in lard, Emulsifying Ointment B.P. and water, as a 5 per cent carboxymethylcellulose gel.

sulphate and so facilitating penetration to the dermal blood supply since passage through the sebum, a mixture of triglycerides, free fatty acids, unsaponifiable matter and cholesterol, at the bases of the follicles may be simplified.

In the present experiments maximum absorption occurred when the drug was presented to the intact skin in an oil phase. Accepting the view that penetration takes place by way of the appendages and that the final barrier to the dermal blood supply is lipoid in nature[9,10], greater absorption would be expected from an oil phase miscible with the skin glycerides and fatty acids and not repelled by this barrier. It may be further postulated that if the mechanism of absorption through the skin depends on the partitioning of the drug across this barrier[11], drugs which are lipid soluble will be absorbed more rapidly than those which are lipid insoluble[12]. The low levels of absorption from water, as a 5 per cent carboxymethylcellulose gel, support the view that absorption through intact rabbit skin is enhanced when the drug is presented to the skin in an oil phase.

These results reinforce the conclusions drawn previously that the intact healthy skin of the rabbit presents a very effective barrier to the passage of drugs applied percutaneously.

Acknowledgements. The authors wish to express their thanks to Mr. J. C. Eaton, M.A., for his assistance with the statistical analysis, and to Miss R. Laird for technical help. One of us (J. C. M.) thanks the Cross Trust for a grant during the tenure of which the above work was done.

REFERENCES

1. Gemmell and Morrison, *J. Pharm. Pharmacol.*, 1957, **9**, 641.
2. Gemmell and Morrison, *ibid.*, 1958, **10**, 167.
3. Woodward, Wright, Everson, Offner, Kramer and Jenner, *Fed. Proc.*, 1944, **3**, 87.
4. Smith and Talbot, *Brit. J. exp. Path.*, 1950, **31**, 65.
5. King and Haslewood, *Lancet*, 1942, **1**, 207.
6. Cumming and Kay, *Quantitative Chemical Analysis*, Gurney and Jackson, London, 1942.
7. Strakosch, *Arch. Derm. Syph.*, 1943, **47**, 16.
8. Strakosch, *ibid.*, 1943, **48**, 384.
9. Burgi, *Schweiz, med. Wschr.*, 1937, **67**, 433.
10. Starkenstein and Hendrych, *Arch. f. exp. Path. Pharmak.*, 1936, **182**, 664.
11. Brown and Scott, *J. Pharmacol.*, 1934, **50**, 373.
12. Kahlenberg, *J. biol. Chem.*, 1924, **62**, 149.