THE PERCUTANEOUS ABSORPTION OF SULPHANILAMIDE

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A test has been designed suitable for the comparison of vehicles and bases as "carriers" of sulphanilamide through the intact skin of rabbits. The blood levels attained were taken as a measure of percutaneous absorption. Statistical analysis of the results showed that the differences in efficiency of the vehicles and bases tested were highly significant.

We have recently reviewed the methods which have been adopted for the evaluation of drug release from topical applications[1]. The sulphonamides penetrate the intact healthy skin only slowly and the amount absorbed into the bloodstream is small. The value of the *in vitro* methods which have been suggested for the estimation of drug release is doubtful since no membrane, either artificial or natural, exists which will simulate the properties of the intact healthy skin.

A number of *in vivo* methods which have been proposed are of interest. Strakosch and Clark[2] applied various sulphonamide ointments to guinea pigs and carried out biopsies on the treated skin. They were unable to demonstrate significant differences between different types of ointments and showed that an increase in concentration of sulphanilamide from one per cent to ten per cent did not greatly increase the concentration found in the skin, although the duration of application did so. Also, an increase in concentration of sulphanilamide over one per cent had little effect on tissue levels. A concentration of up to 6 mg. per cent sulphanilamide was obtained in skin biopsies. Repeated applications of a five per cent sulphathiazole ointment over half the body surface of infants under treatment for skin infections were shown to give blood concentrations of 2 to 4 mg./100 ml.[3]. Zondek, Bromberg and Shapiro went so far as to suggest that the percutaneous absorption of sulphanilamide might be useful where the oral route of administration is not possible[4]. Using rabbits in their experiments they obtained blood concentrations of sulphanilamide of 1 mg./100 ml. in one hour to 8 mg./100 ml. in three and four hours from 0·5 g. sulphanilamide applied as a solution in acetone, glycerol, liquid soap and water. Woodward and others[5] determined sulphathiazole levels in blood samples, catheterised urine samples, at intervals after the application of ointment to the clipped intact skin of rabbits, and carried out localisation tests by analysing definite areas of skin. Among the observations made was one that localisation from a five per cent sulphathiazole ointment equalled that from one of twenty per cent. Clark, Strakosch and Nordlum[6], by the use of iontophoresis, attempted to increase the penetration of sulphonamides into the skin. They found that penetration was essentially equal by iontophoresis and from wet dressings but was less from an ointment base. Fuller, Hawking, and Partridge[7] determined the rate of absorption from standard wounds

in rabbits by estimating the excretion of drug in urine, but they did not concern themselves with the intact skin. Strakosch and Clark[8] and Clark[9], by means of tissue analysis, determined the rate of penetration of sulphonamides into the intact skin of guinea pigs and were unable to show correlation of penetration and the type of ointment base used. Increase in time of application increased penetration but the addition of a solubilising and a wetting agent did not.

In view of the somewhat conflicting and inconclusive results relating to the percutaneous absorption of the sulphonamides, and in spite of the suggestion by Hawking and Lawrence[10] that for all practical purposes the action of the sulphonamides applied to the skin is limited to the surface and superficial layers, this study was undertaken to determine the extent, and to compare the degree, of absorption of sulphanilamide from a number of different vehicles and common ointment bases.

The most promising method for this work was considered to be a technique whereby sulphanilamide, following its cutaneous application, was detected in the bloodstream of the animal. This method provided conclusive proof of its absorption since the sulphanilamide applied in a suitable vehicle must necessarily penetrate the skin and enter the bloodstream in order to be detected and simple penetration or lodgement within the appendages may be discounted. The blood level of sulphanilamide estimated at suitable time intervals would allow a comparison of the efficiency of various vehicles and ointment bases as "carriers" for the drug and since the study of blood concentrations has been a feature of sulphonamide therapy, and the sulphonamide drugs are uniformly distributed in the animal body, this seemed to be the logical approach.

Previous workers in this field have in general provided insufficient results for complete statistical examination. The series of experiments recorded in this paper were designed with a view to obtaining sufficient results to make such an analysis.

Methods

Choice of Sulphanilamide

Sulphanilamide, *p*-aminobenzenesulphonamide, was chosen as a rapidly absorbed sulphonamide with a solubility in water of 400 mg./100 ml. at 15°, and 1,500 mg./100 ml. at 37°, and a solubility in serum of 1,970 mg./100 ml. It is soluble in ethanol, acetone, glycerol, hydrochloric acid and sodium and potassium hydroxides. It is insoluble in ether and benzene and soluble in chloroform to the extent of 25 mg./100 ml. and in propylene glycol to the extent of 10,000 mg./100 ml. The acid dissociation constant (pKa) is 10·43.

Choice of Vehicle

Seven common constituents of ointment bases were tested for their ability to modify the absorption of sulphanilamide and their respective efficiencies were compared. They were liquid paraffin, white soft paraffin, lard, woolfat, propylene glycol, and water in the form of a five per cent carboxymethyl cellulose gel.

Four ointment bases were tested. They were Hydrous Ointment B.P., a water-in-oil emulsion, Emulsifying Ointment B.P., and two oil-in-water emulsions, Hydrous Emulsifying Ointment B.P., and a cetomacrogol emulsifying wax base of the following composition: Cetomacrogol Emulsificans B.P.C. 14 g., liquid paraffin 8 g., and water to 100 g.

Concentration of Sulphanilamide

Although concentration is stated to increase penetration, it has been reported that an increase in strength above five per cent is not significant.

TABLE I

COMPARISON OF PERCUTANEOUS ABSORPTION OF SULPHANILAMIDE IN THE RABBIT FROM DIFFERENT VEHICLES AND BASES

	Total amount sulphanilamide observed in the blood of 6 individual rabbits over 8 hours (each value is the sum of sixteen samples), mg./100 ml.						Total amount sulphanilamide observed in 6 rabbits over 8 hours
	Rabbit number						
	1	2	3	4	5	6	
Liquid paraffin	9·39	9·53	7·71	5·53	6·21	6·32	44·69
White soft paraffin	11·57	11·72	12·02	10·02	9·65	8·85	63·83
Woolfat	8·08	6·84	8·00	6·06	5·61	5·24	39·83
Lard	14·14	16·05	14·54	16·24	13·03	11·92	85·92
Ethyl oleate	12·98	15·60	8·92	6·53	7·89	11·12	63·04
Propylene glycol	17·17	12·19	8·64	8·69	8·80	9·55	65·04
5 per cent methyl cellulose	4·46	4·47	4·64	7·67	7·19	5·93	34·36
Hydrous Ointment B.P.	7·39	12·54	12·29	12·44	9·68	10·56	64·90
Emulsifying Ointment B.P.	7·62	9·08	8·45	6·27	7·71	5·57	44·70
Hydrous Emulsifying Ointment B.P.	5·69	5·72	6·28	6·56	6·58	9·88	40·71
Cetomacrogol emulsifying wax base	8·82	8·30	9·01	8·76	6·97	8·43	50·29

The concentration of sulphanilamide used in all applications was ten per cent weight-in-weight, a strength recommended as a maximum useful concentration[8].

Design of Test

The experiments were designed to give sufficient results for a statistical analysis. All tests were made on six rabbits from the same litter since litter-mates usually yield results less variable than those obtained from animals selected at random from an animal population. Rabbits used in each test consisted of three males and three females between 2·5 and 3·5 kg. weight.

No. of rabbits	Base	No. of tests on each base
6	A	2
6	B	2
6	C	2

The design of the tests made on six rabbits is shown above. The three bases, A, B, and C, were applied in random order thus avoiding any cumulative effect which might have occurred.

Experimental Details

Six Copenhagen White rabbits, litter-mates, weighed and sexed, were used in each test. Food, but not water, was withheld for eighteen hours

before the test since it was found that by this procedure the animals seldom if ever urinated or defaecated while the test was in progress; thus the task of assaying excretory products was avoided. On the day before the test the fur was removed from the skin of the back and sides of each animal with electric clippers, taking care not to damage the skin. The area

TABLE II

COMPARISON OF PERCUTANEOUS ABSORPTION OF SULPHANILAMIDE IN THE RABBIT FROM DIFFERENT VEHICLES AND BASES

	Mean blood levels of sulphanilamide observed in 6 individual rabbits over 8 hours, mg./100 ml.						Mean blood level of sulphanilamide observed in 6 rabbits over 8 hours, mg./100 ml.
	Rabbit number						
	1	2	3	4	5	6	
Liquid paraffin	0·59	0·59	0·48	0·35	0·39	0·39	0·47
White soft paraffin	0·72	0·73	0·75	0·63	0·60	0·55	0·66
Woolfat	0·50	0·43	0·50	0·38	0·35	0·33	0·41
Lard	0·88	1·03	0·91	1·02	0·82	0·75	0·89
Ethyl oleate	0·81	0·98	0·56	0·41	0·49	0·70	0·66
Propylene glycol	1·07	0·76	0·54	0·54	0·55	0·60	0·68
5 per cent methyl cellulose	0·28	0·28	0·29	0·48	0·44	0·37	0·36
Hydrous Ointment B.P.	0·46	0·78	0·77	0·78	0·60	0·66	0·68
Emulsifying Ointment B.P.	0·48	0·56	0·53	0·39	0·48	0·35	0·47
Hydrous Emulsifying Ointment B.P.	0·36	0·36	0·39	0·41	0·41	0·62	0·42
Cetomacrogol emulsifying wax base	0·55	0·52	0·56	0·55	0·44	0·53	0·52

under test was marked by a dermograph pencil using a 6 × 4 inch template placed on the dorsal thoracico-lumbar region of the rabbit. The rabbits were then placed in restraining boxes to prevent any oral absorption and a 0·2 ml. blood sample was withdrawn from the marginal ear

TABLE III

COMPARISON OF PERCUTANEOUS ABSORPTION OF SULPHANILAMIDE IN THE RABBIT FROM DIFFERENT VEHICLES AND BASES

	Table of efficiencies	
	Total amount of sulphanilamide absorbed by six rabbits, mg.	Mean individual rabbit response, mg.
Lard	85·92	14·32
Propylene glycol	65·04	10·84
Hydrous Ointment B.P.	64·90	10·82
White soft paraffin	63·83	10·64
Ethyl oleate	63·04	10·51
Cetomacrogol emulsifying wax base	50·29	8·38
Emulsifying Ointment B.P.	44·70	7·45
Liquid paraffin	44·69	7·45
Hydrous Emulsifying Ointment B.P.	40·71	6·79
Woolfat	39·83	6·64
5 per cent methyl cellulose	34·36	5·73

vein of each animal as a blank for assay purposes. About 30 g. of vehicle or base, containing ten per cent sulphanilamide, was applied to the marked area and inuncted for a period of three minutes. Liquid preparations were applied with a brush. Reapplications, in both instances, were at fifteen minute intervals. At intervals of thirty minutes, after the initial

application, over a period of eight hours, 0·2 ml. blood samples were withdrawn. Thus each completed test involved the withdrawal of sixteen blood samples from each of the six rabbits giving a total of ninety-six samples for one base, and for the complete experiment on three bases, each applied twice, a total of five hundred and seventy-six blood samples. On completion of a single test, the rabbits had the application removed and the area thoroughly washed with warm, soapy water. After several washings, their backs were rinsed and dried. Before a further test, the rabbits were allowed at least one week's rest and their blood was then tested for absence of sulphanilamide.

Estimation of Sulphanilamide in Blood Samples

Sulphanilamide in blood was estimated by King's micro-modification of the method of Bratton and Marshall[11]. All the solutions were assayed at 540 mμ, the wavelength of maximal absorption for sulphanilamide[12].

RESULTS

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

In Table I the results are shown for six individual rabbits; the values obtained in each of the sixteen blood samples withdrawn over the eight

TABLE IV

ANALYSIS OF VARIANCE

Source of variance	Sum of squares	Degrees of freedom	Variance
Between vehicles or bases ..	397·834	10	39·7834
Residual	214·332	55	3·8969

$$F = \frac{39{\cdot}7834}{3{\cdot}8969} = 10{\cdot}21$$

F = 3·7 corresponds to P = 0·001.

The Standard Deviation corresponding to the residual variance of 3·8969 is 1·97.
This gives a Standard Deviation for means of samples of six equal to 0·81.

$$\frac{1{\cdot}97}{\sqrt{6}} = 0{\cdot}8059 \simeq 0{\cdot}81.$$

hours were added together and this sum was entered in the Table. The total amount absorbed by each rabbit can thus be seen. These individual totals were then added together and the response of the six rabbits to the base under examination was found.

Table II was calculated from Table I. The total amount absorbed by the individual rabbits over the eight hours was divided by sixteen, i.e. the number of blood samples withdrawn, and the mean blood level for the duration of the test obtained. The average of the blood levels found for the individual rabbits was then taken for each vehicle and base and this figure was used as an index of the efficiency of the particular vehicle or base tested. These values are represented graphically in Figure 1. Figure 2 represents graphically the mean blood level over eight hours found in the individual rabbits for each of the bases tested.

Table III was also obtained from Table I by dividing the total amount absorbed by the six rabbits over the eight hours by six, thus giving the mean value of an individual rabbit response.

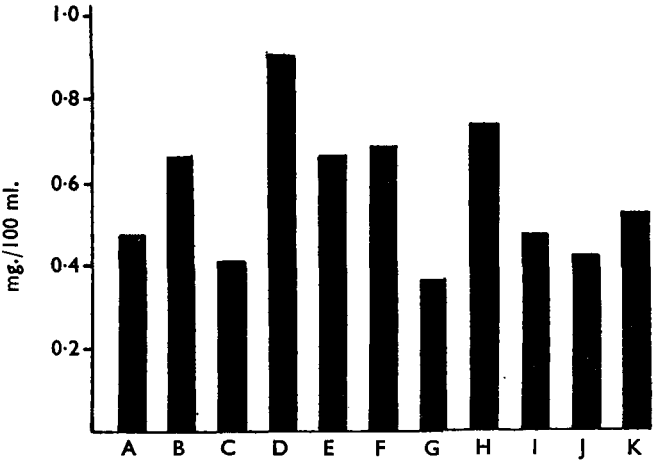

FIG. 1. The mean blood level of sulphanilamide observed in rabbits following the application of the selected vehicles and bases.

A Liquid paraffin.
B White soft paraffin.
C Woolfat.
D Lard.
E Ethyl oleate.
F Propylene glycol.
G 5 Per cent methyl cellulose gel in water.
H Hydrous Ointment B.P.
I Emulsifying Ointment B.P.
J Hydrous Emulsifying Ointment B.P.
K Cetomacrogol emulsifying wax base.

FIG. 2. The blood level of sulphanilamide observed in individual rabbits following the application of the selected vehicles and bases. Six animals were used with each base. See Figure 1 for key.

Table IV shows the analysis of variance carried out on the results of the series of experiments. The Standard Deviation corresponding to the residual variance of 3·8969 is 1·97 giving a standard deviation for means of samples of six equal to 0·81. This value may be used as a rough measure for significant differences between the vehicles and bases in Table III. A difference between the vehicles and bases in the mean individual rabbit response of less than 0·81 is unlikely to be significant. A difference in this response of more than 0·81 is likely to be significant. It will be seen from Table III that the vehicles and bases fall into six groups. the differences between each group being significant.

Discussion

A statistical analysis of the experimental results has been made. An analysis of variance of the results in Table I showed that the differences in the bases as efficient "carriers" of sulphanilamide were very highly significant ($F = 10{\cdot}21$ where $F = 3{\cdot}7$ for $P = 0{\cdot}001$). On this basis it is possible to arrange the vehicles and bases in several groups. Within a group, the vehicles or bases are equally efficient, but there are significant differences between the vehicles or bases in different groups. The difference in response between individual rabbits was insignificant.

Sulphanilamide, when applied in various vehicles and bases to the intact, healthy skin of rabbits, penetrates the skin and is absorbed into the blood stream where it can be detected in measurable quantities. The amount of sulphanilamide circulating in the bloodstream is small. For example, using lard, which was found to be the most effective vehicle or "carrier," the maximum blood level observed in any one rabbit was 1·03 mg./100 ml. and from the six rabbits tested with this vehicle the mean blood level obtained was 0·89 mg./100 ml. The blood levels obtained from other vehicles and bases were lower and in the case of the five per cent carboxymethyl cellulose gel in water, which was in effect absorption from water, the methyl cellulose being present merely as an aid to application, a mean blood level of 0·36 mg./100 ml. was obtained. For the other vehicles and bases, the mean blood levels varied between 0·41 and 0·66 mg./100 ml.

These findings emphasise that the intact healthy skin of the rabbit presents an effective and almost impermeable barrier to the passage of sulphanilamide applied percutaneously, especially when it is considered that approximately one-fifth of the body area of the animals under test was exposed. Blood levels which have been reported over 3 mg./100 ml. following the application of various topical preparations containing sulphanilamide should be viewed with caution, but in the case of wounded, burned, or diseased skin, where the outer epithelial layers are damaged, systemic absorption is greater and blood levels of this order may be expected. It is interesting to note that for prophylaxis the blood level of sulphanilamide considered adequate is 2 to 3 mg. per 100 ml.[10].

Despite the small amount of sulphanilamide absorbed, differences in the efficiencies of the chosen vehicles and bases as "carriers" could be

clearly distinguished and the method can, therefore, be used for comparative purposes.

Absorption from the methyl cellulose gel showed that water as a "carrier" of sulphanilamide was the least effective vehicle tested. This was borne out by the low absorption from Hydrous Emulsifying Ointment B.P., an oil-in-water emulsion, which has a continuous water phase, whereas Hydrous Ointment B.P., a water-in-oil emulsion, with a continuous oil phase, was approximately one-and-a-half times more efficient as a "carrier." Such a finding had been reported previously[13] and suggests that the absorption of sulphanilamide by way of the sebaceous glands and hair follicles is simplified when the drug is presented to the skin in an oil phase. The skin surface being protected by a greasy layer of waxes is impervious to water and aqueous solutions and absorption of sulphanilamide occurring from the aqueous phase must be due to the partitioning of the drug between the aqueous phase and the fats of the sebaceous glands and hair follicles.

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