THE PENETRATION OF PHENYLMERCURIC DINAPHTHYLMETHANE DISULPHONATE INTO SKIN AND MUSCLE TISSUE

By A. A. GOLDBERG, M. SHAPERO AND E. WILDER

From the Research Laboratories, Messrs, Ward, Blenkinsop & Co. Ltd., Shepton Mallet, Somerset

Received November 1, 1949

DURING recent years various crystalloid salts of phenylmercuric hydroxide, notably the nitrate and acetate, have attained wide clinical use for the treatment of infections of the skin [1,2,3,4,5,6]. Although these salts have an extraordinarily high antibacterial activity which is not significantly impaired by the presence of serum, pus and tissue debris [3,7] yet they possess little or no capacity for penetration of and substantive fixation to skin and muscle.

The present communication relates to a colloidal salt of phenylmercuric hydroxide which, by virtue of the surface active and hydrotropic properties of the colloidal anion, rapidly penetrates living skin and fixes itself substantively to the subdermal connective tissue. The advantages of penetrative and protein substantive properties in a bactericide for topical dermatological application are twofold. The reagent penetrates to the site of organisms which have invaded the skin and hair follicles in depth and builds up a high concentration in these regions ; the irreversibility of the adsorption process retards absorption of the organomercurial into the general circulation.

Phenylmercuric dinaphthylmethane disulphonate is a white amorphous salt containing 40·8 per cent. of organically bound mercury. Although almost insoluble in water it rapidly dissolves in aqueous solutions of alkali metal dinaphthylmethane disulphonates to yield stable colourless solutions which may be buffered to any desired *p*H value. In these solutions the solutes are highly ionised and behave as typical colloidal electrolytes. Colloidal electrolytes possess two properties by virtue of which they influence biological activity, viz., their strong tendency to adsorb at interfaces and their power to form charged hydrated colloidal aggregates (micelles). Micelles, owing to their structure, adsorb ions carrying a charge opposite to that upon the micelle and in addition are able, by the process of molecular adsorption, to solubilise many organic substances which are normally insoluble in water. Micellar solubilisation is known to facilitate transport of sparingly soluble substances at a rate which may be enormously greater than the normal process of solution and diffusion. Unless otherwise stated the solution used in the present work contained 0·1 per cent. of phenylmercuric dinaphthylmethane disulphonate, 1·9 per cent. of potassium dinaphthylmethane disulphonate and 1·0 per cent. of the potassium dihydrogen phosphate-disodium hydrogen phosphate buffer system in water at *p*H 7·0.

Two methods have been employed for the determination of the extent of penetration after application to skin and muscle. Sections were made in planes perpendicular to the plane of penetration and these sections developed histochemically in order to visualise the area containing

phenylmercuric ion ; sections were made in planes parallel to the plane of penetration and submitted to quantitative chemical analysis for mercury.

Penetration of Phenylmercuric Dinaphthylmethane Disulphonate into Dead Muscle Tissue

Longissimus dorsi muscles, from freshly killed rabbits, were cut into cubes having *ca.* 15 mm. edges and these were immersed in the 1 : 1000 solution at 20°C. for 24 or 48 hours. The treated cubes were washed in running water for 6 hours in order to remove excess of reagent and then immersed in isotonic formol-saline for 24 hours. After washing for 24 hours in running water the cubes were embedded in gelatin and 40μ sections cut on the carbon-dioxide freezing microtome. Sections were developed in order to visualise (a) phenylmercuric ion and (b) dinaphthylmethane disulphonate ion. (a) The sections were dried between filter papers and flooded for 5 minutes on slides with a freshly made 0·2 per cent. carbon tetrachloride solution of dithizone. They were then immersed for 3 minutes in 10N aqueous ammonia and finally left floating in 2N ammonia overnight. This procedure removes excess of dithizone, leaving the insoluble phenylmercuric complex of dithizone as a deep brown peripheral margin surrounding a white central area. (b) The freshly cut wet sections were flooded for 3 minutes with a 0·5 per cent. aqueous solution of crystal violet, washed for 15 minutes in running water, immersed in 1 per cent. w/v sulphuric acid for 15 minutes and then left floating in a large volume of water overnight to remove excess of the stain. The insoluble dinaphthylmethane disulphonic acid salt of the crystal violet base remains as a bright purple peripheral margin surrounding the colourless central area of the section.

The depth of penetration of each ion into the muscle was measured by taking 10 readings from each of 10 sections, the mean value being recorded. The effect of the cation of the solubilising salt upon the depth of penetration is shown in Table I : all the solutions contained 0·1 per cent. of phenylmercuric dinaphthylmethane disulphonate and 1·9 per cent. of the alkali metal dinaphthylmethane disulphonate (solubilising salt) and 1·0 per cent. of the phosphate buffer system at *p*H 7·0 (Fig. 1).

TABLE I

Solubilising Salt cation	Penetration of Phenylmercuric ion		Penetration of Dinaphthylmethane disulphonate ion	
	24 hours	48 hours	24 hours	48 hours
Sodium	0·40 mm.	0·66 mm.	0·42 mm.	0·82 mm.
Ammonium	0·48 mm.	0·78 mm.	0·44 mm.	0·90 mm.
Lithium	0·51 mm.	0·95 mm.	0·58 mm.	1·18 mm.
Potassium	0·52 mm.	1·04 mm.	0·62 mm.	1·20 mm.

The depth of penetration in all cases was substantially the same with solutions at *p*H values of 6·0, 7·0 and 8·0 ; at *p*H 4·0, however, there was less penetration.

Other muscle cubes immersed in the same solution for 48 hours at 20°C. were washed by immersion for 6 hours in slowly stirred distilled water and then mounted in gelatin in a metal mould in order to preserve the original cubic shape. Layers 1 mm. thick were removed from 5 faces of the cube and the remainder of the cube sectioned in a plane parallel to the remaining 6th surface, commencing from this surface. Sections of 80μ thickness were cut, these burnt with sulphuric acid and hydrogen peroxide to destroy all organic matter and the resulting mercuric sulphate determined by chemical analysis. The weight of each section was computed from its physical dimensions and its density, the density of the muscle being determined by the method of flotation using normal cubes and aqueous solutions of sodium chloride of varying specific gravity.

Typical results from separate determinations with muscle taken from three different rabbits I, II and III are shown in Table II. In the case of muscle cubes from rabbit No. III, the cubes IIIa, after treatment, were given the standard wash of 6 hours while the cubes IIIb were given a prolonged wash in large volumes of repeatedly changed distilled water for 48 hours in order to determine the extent of fixation of the compound to the muscle tissues, i.e. the degree of irreversibility of the adsorption process.

TABLE II

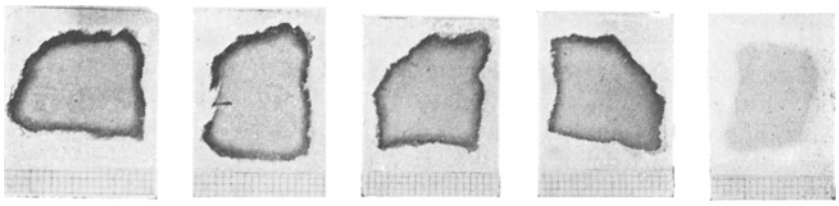
Section	Hg Found (Micrograms)				g. of compound per kg. of Tissue			
	I	II	IIIa	IIIb	I	II	IIIa	IIIb
1st (Top)	110	274	220	204	14·2	14·1	12·7	10·0
2nd	73	245	160	144	9·4	12·6	9·3	7·1
3rd	54	—	118	100	7·0	—	6·8	4·9
4th	43	—	110	98	5·6	—	6·3	4·8
5th	42	104	96	96	5·4	5·4	5·6	4·7
6th	35	—	88	90	4·5	—	5·2	4·4
7th	34	—	76	74	4·4	—	4·3	3·6
8th	30	81	66	72	3·9	4·2	4·2	3·5
9th	28	—	63	66	3·6	—	3·8	3·2
10th	24	78	52	60	3·1	4·0	3·5	2·9

Area of sections : I, 16 × 13 mm. ; II, 29 × 18 mm. ; IIIa, 31 × 15 mm. ; IIIb, 25 × 22 mm. Thickness of sections : 0·080 mm. Muscle density : 1·14 g./c.c.

Cubes of muscle tissue immersed for 48 hours in 1 : 1000 aqueous phenylmercuric acetate and then given standard 6 hours rinse prior to sectioning showed, by the histochemical method, a penetration in low concentration to a depth of 0·28 mm. When the intact muscle cubes were given a prolonged wash of 24 hours the sections, after developing with dithizone, appeared completely white with no peripheral margin showing complete absence of phenylmercuric ion. This was confirmed by the method of quantitative chemical analysis.

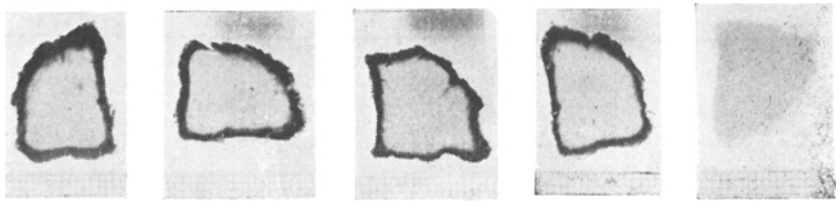
Two important properties of phenylmercuric dinaphthylmethane

disulphonate present themselves from Table II : (i) it is substantive to muscle tissue, i.e. the compound is adsorbed upon the tissue to build up a concentration in the latter greater than that in the bathing solution, (ii) the adsorbate is strongly resistant to removal by washing i.e. the adsorption process is not easily reversible. The low penetration of phenylmercuric acetate and its complete removal from the cubes by 24 hours washing presents evidence that the penetrating and substantive properties must be associated with the presence of the colloidal anion.



*p*H 4·5 *p*H 6·0 *p*H 7·0 *p*H 8·0 Control

Penetration of Phenylmercuric Ion.



*p*H 4·5 *p*H 6·0 *p*H 7·0 *p*H 8·0 Control

Penetration of Dinaphthylmethane Disulphonate Ion.

FIG. 1. Penetration of Phenylmercuric dinaphthylmethane disulphonate into rabbit muscle. Small squares are equivalent to 1 sq. mm.

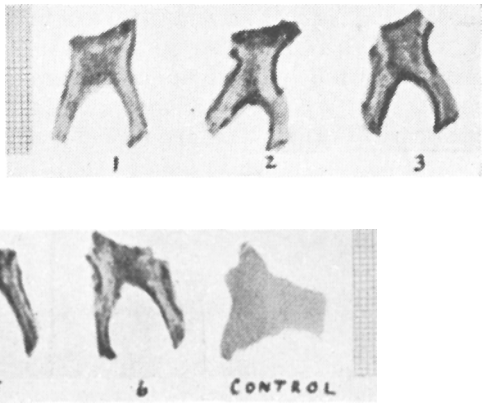
PENETRATION OF PHENYLMERCURIC DINAPHTHYLMETHANE DISULPHONATE THROUGH LIVING SKIN INTO SUBCUTANEOUS CONNECTIVE TISSUE

The following is typical of experiments performed many times. An area of *ca.* 5 sq. cm. of a rabbit's back was shaved and the skin painted with the 1 : 1000 solution 3 times per day for 3 days. At the end of this time the skin was healthy, elastic to the touch and showed no sign of scaling or irritation. The animal was killed, the treated area dissected to a depth of *ca.* 1·5 cm. and this, after washing for 3 hours in water, cut into cubes in such a manner that one face of each cube consisted of the original skin. The cubes were embedded without distortion in a metal mould and frozen on the microtome stage with the skin uppermost and perfectly horizontal : sections (a) of 40μ thickness were made of the skin in a plane parallel to the surface of the latter. Other cubes were mounted on the microtome with the skin surface vertical and sections

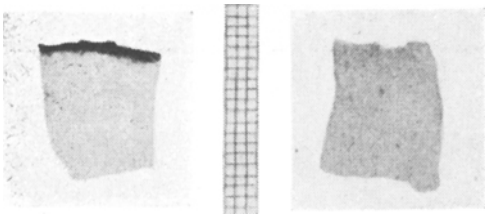
(b) made in planes perpendicular to the surface of the skin. Sections (a) were stained and developed to show penetration and fixation of phenylmercury ion and also analysed for mercury content. Sections (b) after removing the thin strip of skin were stained and developed to show penetration of the reagent into, and fixation upon, the subcutaneous connective tissue. In all, 50 slides were examined in order to record depths of penetration.

The results are shown photographically in Fig. 2. The phenylmercury ion penetrates through the epidermis, the papillary and reticular layers of the corium and the subcutaneous adipose connective tissue—a total

A. SKIN. Consecutive 40 μ sections cut parallel to surface of skin (skin surface is No. 1).

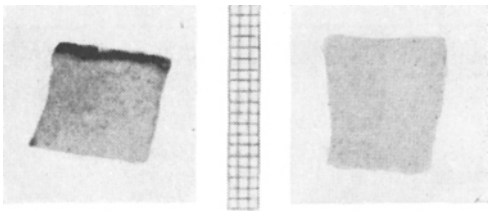


B. SUBDERMAL CONNECTIVE TISSUE. Sections cut perpendicular to surface of skin: the skin, 900μ thick, is not shown.



CONTROL

C. MUSCLE. Sections cut perpendicular to muscle surface. Squares are equivalent to 1 sq. mm.



CONTROL

FIG. 2. Penetration of phenylmercuric dinaphthylmethane disulphonate through living skin into subdermal tissue and into exposed living muscle after treatment with a 1 in 1000 solution applied three times a day for three days. All the sections were developed with dithizone to show the fixation of the phenylmercuric ion.

in the rabbit of *ca.* 1500μ—and then for *ca.* 700μ into the subdermal muscular tissue, the total penetration being of the order of 2·2 mm. There is a heavy concentration of the reagent in and around the hair follicles.

In order to determine quantitatively the depth of penetration through living skin into subdermal tissue an area of *ca.* 10 cm.×5 cm. of the lateral dorsal region of several rabbits was shaved. Lint dressings soaked in the 1 : 1000 solution were applied, these covered with oiled skin and cotton wool, and secured by bandaging ; a fresh soaked dressing was applied each morning for 3 consecutive days The animals were killed, the treated areas dissected to a depth of *ca.* 1·5 cm. and cubes of the muscles with the attached skin cut in the same manner as described above. The skin was stripped off the cubes, burnt with sulphuric acid and perhydrol, and the total mercury content assayed. The average thickness of the skin was computed from its area, weight and density, the density being determined by the method of flotation. The subdermal muscle was sectioned in planes parallel to the plane of the skin surface and the total mercury determined in each section. Table III records the results found in two typical experiments with two adult rabbits.

TABLE III

Tissue	Hg found (micrograms)		g. of compound per kg. of tissue	
	(a)	(b)	(a)	(b)
Skin	68	64	0·29	0·28
Subdermal Muscle				
1st Section	41	37	2·8	2·8
2nd „	38	36	2·6	2·7
3rd „	34	32	2·3	2·4
4th „	24	29	1·6	2·2
5th... „	18	20	1·2	1·5
6th... „	12	16	0·8	1·2
7th... „	8	10	0·5	0·7

Area of skin and Sections (a) 25×16 mm. ; (b) 24×15 mm. Thickness of skin : (a) 1·41 mm. ; (b) (b) 1·50 mm. Density of skin : 1·03 g./c.c. Thickness of Muscle Sections : 0·080 mm. Density of Muscle : 1·14 g./c.c.

It is apparent that phenylmercuric dinaphthylmethane disulphonate passes through the living skin and enters deeply into the subdermal connective tissue and muscle ; at the 6th section of the latter, a depth of *ca.* 2·0 mm. below the surface of the skin, the concentration of the adsorbed substance is *ca.* 1 g./kg., i.e. the concentration of the compound in the solution that was applied. At the depth of the first section of the muscle, *ca.* 1·5 mm. below the skin surface, the concentration of the adsorbed substance is of the order of three times that existent in the applied solution.

PHENYLMERCURIC DINAPHTHYLMETHANE DISULPHONATE

Systemic Toxicity

Phenylmercuric dinaphthylmethane disulphonate solution was administered to 20 g. mice, in groups of 4 by the oral and the intraperitoneal route. Because of the well known delay in the appearance of toxic symptoms after the ingestion of heavy metal salts the animals were observed for 14 days before recording results.

Oral: LD100 80 mg./kg. ; LD50 70 mg./kg. ; LD0 50 mg./kg.

Intraperitoneal: LD100 50 mg./kg. ; LD50 25 mg./kg. ; LD0 10 mg./kg.

Guinea-pigs, in groups of 2, were given single oral doses of 5, 10, 20, 30, 40 and 50 mg./kg. of phenylmercuric dinaphthylmethane disulphonate and then kept on a diet of oats and fresh vegetables : the average initial weight of the animals was 300 g. After 6 weeks one animal which had received 50 mg./kg. was killed and the organs examined. The liver and kidneys were of normal size but contained numerous hæmorrhages and necrotic patches ; sections of both organs after development with dithizone showed deposits of mercury. The spleen was of normal size ; there were no histological changes but mercury deposits were present. At the end of 3 months the other 11 animals appeared normal and their average weight had increased to *ca.* 450 g. On autopsy no macroscopic or microscopic change was visible in the liver, kidneys or spleen. Sections of these organs developed with dithizone in all cases gave a negative test for mercury. (This reagent detects a concentration of mercuric, mercurous or phenylmercuric ion in section tissue of 1 mg./kg.)

Tissue Toxicity

Muscle cubes from freshly killed rabbits which had been immersed in solutions containing 0·1 per cent. of phenylmercuric dinaphthylmethane disulphonate plus 1·9 per cent. of potassium dinaphthylmethane disulphonate at *p*H values ranging between 6·0 and 8·0 for 48 hours at 20°C. maintained their original texture and retained the softness and elasticity of fresh normal muscle. Muscle thus treated is digested by pepsin and by trypsin almost to the same extent as untreated muscle. Complete gastrocnemius muscles of rabbits treated in this manner retained 90 per cent. of their normal elasticity as recorded on the kymograph.

Rabbit intestine which had been immersed in the 0·1 per cent. solution at *p*H 7·0 for 24 hours was found to be freely permeable to neutral red, glucose and fructose although rather less so than normal intestine.

Influence on the Opsono-Phagocytic Index.

Tubes containing 0·05 ml. of an emulsion of living *Streptococcus pyogenes*, 0·05 ml. of a suspension of washed rabbit leucocytes, 0·01 ml. of normal rabbit serum and varying amounts of solution were incubated for 15 minutes at 37°C. Slides were prepared and 50 polymorphonuclears examined for ingested bacteria.

TABLE IV

0·1 per cent. Solution	Number of Ingested Bacteria	Opsonic Index
ml.		
nil	200	4·0
0·01	185	3·7
0·025	174	3·5
0·05	160	3·2

Bactericidal and Antimycotic Activity of Phenylmercuric Dinaphthylmethane Disulphonate

The potentiating effect of the colloidal dinaphthylmethane disulphonic ion upon the bacteriostatic activity of the phenylmercuric ion in media containing up to 80 per cent. concentrations of serum has been reported in a previous communication[7]. The bactericidal activity recorded below is the time taken in minutes for the 1 : 1000 solution to effect sterilisation of a 24 hour subculture ; this time is given in parentheses after each organism. The organisms were grown in double strength broth for 24 hours and then an equal volume of 1 : 500 solution added and the solutions rapidly mixed. At intervals of 5 minutes a loopful was removed and inoculated into 50 ml. of fresh broth which was then incubated for 48 hours at 37°C.

Staphylococcus aureus (25), *Escherichia coli* (25), *Bacillus subtilis* (30), *Pseudomonas pyocyaneus* (20), *Bacillus proteus* (20), *Staphylococcus citreus* (25), *Staphylococcus albus* (25), *Streptococcus fæcalis* (20), *Pseudomonas fluorescens* (20), *Bacillus mesentericus* (20).

Czapek's medium reinforced with 0·1 per cent. of asparagine was used for the first 6 fungi in Table V, the serial dilutions being incubated at 22°C. for 7 days. Growth in the controls was slow for the first 3 days but then rapidly accelerated ; absence of turbidity was recorded as the maximum inhibitory dilution. In the case of *Monilia albicans* the medium used was Lab-Lemco made up in half strength beer-wort instead

TABLE V

Organism	Maximum Dilution in Multiples of 1000	
	Inhibitory	Sterilising
Rhizopus nigricans	10	—
Penicillium expansum	10	—
Penicillium citrinum	9	—
Penicillium notatum	16	—
Aspergillus fumigatus	7	—
Aspergillus niger	9	—
Monilia albicans	16	—
Microsporon aodouini	24	20
Microsporon lanosum (*felineum*) ...	24	20
Trichophyton mentagrophytes (*gypseum*)	24	20
Trichophyton purpureum	50	32
Epidermophyton inguinale (*floccosum*) ...	50	50

of water. The fungistatic and fungicidal activities against the last 5 dermatophytes were determined in 2 per cent. glucose broth containing 1 per cent. of peptone, the dilutions being incubated at 25°C. for 21 days.

Summary

1. The pharmacology of a bactericidal and mycoticidal colloidal electrolyte, phenylmercuric dinaphthylmethane disulphonate, is described.

2. The compound, applied topically in the form of its aqueous solution, penetrates the living epidermis, dermis and connective adipose tissue, entering into the subjacent muscle. The electrolyte is substantive to protein tissue, i.e., it is adsorbed upon living muscle to build up a higher concentration in the latter than that which exists in the bathing solution. The adsorption process is not easily reversible.

3. Phenylmercuric dinaphthylmethane disulphonate solutions do not cause any significant biochemical change in body tissue as shown by the macroscopic and microscopic appearance and the elasticity of tensor muscle, the permeability of intestine and the opsono-phagocytic index.

4. The toxicity of phenylmercuric dinaphthylmethane disulphonate is of the order of that of colloidal silver and less than that of silver nitrate.

The authors thank Dr. L. Syms for the antimycotic activities against the five dermatophytes in Table IV.

References

1. Biskind, *Surg. Gynec. Obstet.*, 1933, **57**, 261.
2. Birkhang, *J. Infect. Dis.*, 1933, **53**, 250.
3. Armangue and Maestres, *Arch. Med. Cirug. espec.*, 1934, **37**, 1074.
4. Levine, *J. Amer. med. Ass.*, 1933, **101**, 2109.
5. *U.S. Naval med. Bull.*, 1936, **34**, 527.
6. Council Report, *J. Amer. med. Ass.*, 1934, **102**, 1224.
7. Goldberg, Shapero and Wilder, *J. Pharm. Pharmacol.*, 1950, **2**, 20.