The mechanism of action of the sulfa compounds and probable explanations for their inhibition by various factors are thoroughly discussed in recent papers by Long,[4] by Kohn and Harris,[5] and by Harris and Kohn.[6] Presumably the same reasoning may be applied to paranitrobenzoic acid.

Conclusion. The bacteriostatic effect of paranitrobenzoate on the pneumococcus is markedly reduced by the products of disintegrating tissue.

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Penetration of Sulfonamides Through Intact Skin by Iontophoresis and other Means of Local Application.

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This report presents some preliminary studies in which penetration by topical application of various sulfonamides has been measured by tissue analysis. It was thought that iontophoresis (electrolytic ion transport) might be an effective means of forcing the anion of sulfonamide salts into the skin. Penetration by iontophoresis was, therefore, compared with that from wet dressings and from an ointment, in the rat, rabbit, and human. The effect of circulation and the depth of penetration were estimated.

Methods. The experimental animals were anesthetized with nembutal and the hair removed with clippers. In the iontophoresis experiments, Canton flannel (6 oz grade) was wetted with the test solution and applied to the skin. Metal gauze electrodes were firmly applied over the flannel as the negative pole. The positive electrode was applied to one of the extremities, and currents of 1-2 milliamperes at 110-125 volts D.C. were applied.

Penetration from wet dressings (gauze or flannel) without current passage, was similarly studied in comparable areas on the same animal or on different animals.

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[4] Long, P. H., *Sigma Xi Quarterly*, 1941, **29**, 149.

[5] Kohn, H. I., and Harris, J. S., *J. Pharm. and Exp. Therap.*, 1941, **73**, 343.

[6] Harris, J. S., and Kohn, H. I., *J. Pharm. and Exp. Therap.*, 1941, **73**, 383.

Penetration from an ointment base was also compared. 5% sodium sulfathiazole (NaSAT) in "Aquaphor"* was used in these studies. 5% NaSAT and saturated aqueous (1.8%) calcium sulfathiazole (CaSAT)† were used in the iontophoresis and wet dressing experiments.

At the termination of the application period, biopsies or autopsies were performed after exhaustively washing the skin with distilled water and alcohol. Tissue aliquots were removed from within the areas of applications, washed several times through water and alcohol, blotted, weighed, thoroughly macerated with sand in a mortar, diluted to appropriate volumes to contain 3% trichloracetic acid, and filtered. The filtrates were passed back through the filters 5 times, since the fourth filtrates were found to contain no measurable sulfathiazole (SAT). Recoveries of known amounts of SAT added to tissue were obtained within 1-2%. The animal tissue aliquots weighed 0.5-2.0 g and the human biopsies, 0.2-0.6 g. The latter were performed under novocaine anesthesia by a punch, the wounds healing by secondary intention. Free SAT was colorimetrically determined in the filtrates by the method of Bratton and Marshall.[1]

Results. 1. *Penetration of CaSAT and NaSAT into the intact skin of the rat by iontophoresis and from ointment.* Penetration of a saturated solution of CaSAT (1.8%) from negative electrodes was compared with that of 5% NaSAT ointment applied more distally on the same animal. 30 x 25 mm electrodes were used in iontophoresis. An equal area was covered with the ointment. The rats were sacrificed at various times and skin aliquots were analyzed. The results appear in Table I A. Although comparison is made of two different sulfonamide salts at different concentrations and differently applied, the tissue concentrations found were apparently identical. The higher value for iontophoresis versus ointment after 2 hours may not be significant since too few determinations were made and since individual variations in duplicate determinations are large, as seen in Tables II and III.

2. *Penetration of NaSAT into the intact skin of rats by iontophoresis and from wet dressings and ointment.* These experiments were performed as above, but for longer periods, and using the same

* Duke Laboratories, Inc. Consists of 5% cholesterol in petrolatum. 5 g of NaSAT were suspended in 5 ml of water and worked into the "Aquaphor" with a spatula.

† Kindly supplied by Doctor W. W. Spink, of the Department of Internal Medicine, this institution.

[1] Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, 1939, **128**, 537.

TABLE I.
Penetration of CaSAT and NaSAT into Intact Skin of Rats.
A. Penetration of CaSAT by Iontophoresis compared with that of NaSAT from Ointment.

Treatment	Time applied (hr) No. of rats	Tissue SAT mg% 0.5	1.0	1.5	1.7	2.0
Iontophoresis (Sat'd CaSAT)	5	6	9	11	29	58
Ointment (5% NaSAT)	6	7	9	10	14	27
					40	
					—	
				Av.	27	

B. Penetration of NaSAT by Iontophoresis and from Wet Dressings and Ointment.

Treatment	Time applied (hr) No. of rats	Tissue SAT mg% 2.0	4.0	6.0
Iontophoresis (5% NaSAT)	6	53	108	69
		68	55	77
		—	—	—
		Av. 60	Av. 81	Av. 73
Wet dressing (5% NaSAT)	6	62	106	158
		132	91	110
		—	—	—
		Av. 97	Av. 103	Av. 134
Ointment (5% NaSAT)	1	27	—	—

concentration of the same sulfonamide salt (NaSAT) throughout. Duplicate determinations were made, using 2 animals for each type and time period of application. Tissue aliquots were analyzed after 2, 4, and 6 hours. One ointment determination from Table I A is included. The results are seen in Table I B. Although the individual variations were large, it is apparent that the tissue concentration tended to plateau after 2-3 hours (see also Table I A), at an average concentration of ca 50 times less than that of the applied solutions. No large differences in penetration of SAT salts by iontophoresis and from wet dressings are apparent until 6 hours, when the latter shows better penetration. At 2 hours, penetration from ointment is inferior to that from wet dressings or iontophoresis.

3. *Depth of penetration of CaSAT through skin of rabbit by iontophoresis and from wet dressings.* Areas 6 x 8 cm on the thighs of a rabbit were treated with 5% NaSAT by iontophoresis and by wet dressing for 3 hours. Aliquots of skin, subcutaneous muscle, the deeper muscles, and blood, were analyzed for SAT. The results appear in Table II A. A 40% greater concentration of SAT was obtained in the skin by iontophoresis than by wet dressing, which

TABLE II.

A. Depth of Penetration of CaSAT through Skin of Rabbits by Iontophoresis and from Wet Dressings.
3 hrs application. 6-lb rabbit. Thigh. (Detail in text.)

Treatment	Tissue SAT mg%			
	Skin	Subcut. muscle	Deep muscle (biceps, adductor)	Blood
Iontophoresis (Sat'd CaSAT)	312	64.5	trace	trace
Wet dressing (Sat'd CaSAT)	224	0.7	"	"

B. Penetration of CaSAT and NaSAT into Intact and Ligated Legs of Rats by Iontophoresis and from Ointment.
1.7 hrs application. 2 rats. Thigh. (Detail in text.)

Rat No.	Treatment	Tissue SAT mg%		
		Leg	Skin	Deep muscle (biceps)
1.	Iontophoresis (Sat'd CaSAT)	Left (ligated)....24 Right (intact)....29		trace "
2.	Ointment (5% NaSAT in "Aquaphor")	Left (ligated)....19 Right (intact)....40		" "

we consider a small difference (compare Fig. 1). The iontophoresis seemed to produce a higher concentration in the subcutaneous muscle in the rabbit. Immeasurably small quantities of SAT were found in the deeper muscles and in the blood. Very large concentrations of SAT may be obtained in the intact skin by topical wet application, while the blood level is negligibly small.

4. *Penetration of CaSAT and NaSAT into the intact and ligated legs of rats by iontophoresis and from ointment.* The left legs of 2 rats were tightly ligated and the lateral surface of the left thigh treated with 5% NaSAT ointment in 1 rat, and with saturated aqueous CaSAT in the other. The right thighs acted as controls with intact circulation. Table II B shows the results. Ligation has no effect on the penetration of NaSAT by iontophoresis for 100 minutes, whereas ligation may have caused some impairment in penetration of CaSAT. Again we do not feel that this difference is significant, due to paucity of data and the individual variations seen in the tables. We feel that in general, in the rat, ligation has no effect on penetration (see discussion at end of paragraph 6). The deeper muscle layers contained negligible amounts of SAT.

5. *Penetration of NaSAT into human skin by iontophoresis, wet dressings, and ointment.* 7x7 cm areas were treated for 3 hours with 5% NaSAT by the 3 methods, and duplicate punch biopsies from each area analyzed for SAT. Table II shows the results. No difference in penetration of NaSAT was observed by ionto-

TABLE III.
Penetration of NaSAT into Intact Human Skin.
Normal skin from thigh. Duplicate punch biopsies through the corium. Biopsy weights 30-50 mg. 5% NaSAT applied for 3 hours.

	Tissue SAT mg%		
	Iontophoresis	Wet dressing	Ointment
Case 1 (64 yr female, normal weight)	55	57	trace
	—	45	"
Case 2 (26 yr male, corpulent)	36	33	"
	60	87	"
	—	—	—
	av. 50	av. 55	av. "

phoresis compared with that from wet dressing, and penetration from ointment was negligible. In this latter respect human skin is less permeable than rat skin, although penetration of NaSAT in rat skin from ointment was also less than from wet dressings or iontophoresis.

6. *Penetration of CaSAT and NaSAT into the skin of live and dead rabbits by iontophoresis and from wet dressings.* Penetration of saturated aqueous CaSAT (1.8%) from wet dressings and by iontophoresis, into the skin of the back was studied for 1 and 3 hours in one rabbit; and that of 5% NaSAT by iontophoresis and from wet dressings in one anesthetized and in one dead rabbit. Biopsies were performed after 1, 2, and 3 hours. Fig. 1 depicts the

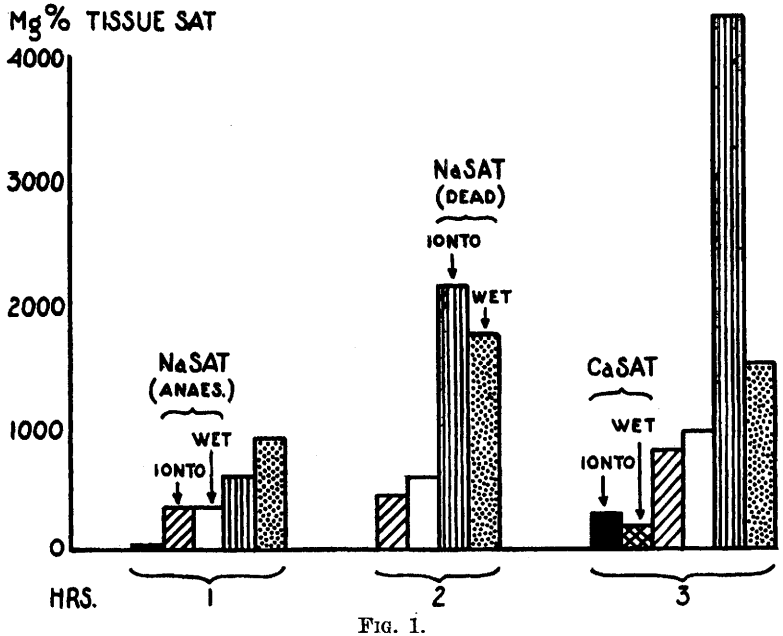

FIG. 1.

results. The skin concentration of SAT after iontophoresis and wet dressing applications of the CaSAT were not noticeably different after 3 hours, and were less than those obtained by similar applications of 5% NaSAT. Slightly higher levels were obtained after 2 and 3 hours' wet dressing application than by iontophoresis in the anesthetized animal, but iontophoresis effected much higher concentrations than wet dressings in the skin of the dead animal after 3 hours, reaching a concentration of 4%. This observation may explain the lack of differences between iontophoresis and wet dressing applications seen in Tables I-III. Electroösmosis of water toward the negative pole may counteract electrical ion transport of sulfonamide anions in the live animal with normal skin permeability and circulation, and in the edematous ligated limbs of rats; where it does not in the dead animal. More experiments than are reported in this preliminary paper will be necessary to firmly establish this point.

A second observation of importance is that within the periods of time studied, NaSAT attains markedly higher concentrations in rabbit skin than in human or rat skin.

Summary. 1. The penetration of sodium sulfathiazole (NaSAT) into the intact skin of rats, rabbits, and humans, up to 6 hours' application by iontophoresis and from wet dressings, is essentially equal, while that from an ointment base is less than either of these. Penetration is somewht better from wet dressings than by iontophoresis after longer times.

2. Saturated (1.8%) calcium sulfathiazole (CaSAT) penetrates the intact skin of rats to the same extent by iontophoresis as from 5% NaSAT ointment.

3. Iontophoresis of NaSAT through the intact skin of rabbits produces a greater concentration of SAT in subcutaneous muscle than wet dressing application, but the concentrations in deep muscle and blood are negligibly small.

4. Impaired circulation in rats by ligation of the limbs, has little effect on penetration of CaSAT and NaSAT into these limbs by iontophoresis and from ointment.

5. Penetration of CaSAT and NaSAT by iontophoresis and from wet dressings into rabbit skin is essentially the same. Penetration of NaSAT into the skin of dead rabbits by iontophoresis is greater than from wet dressings.