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Studies on the Local Treatment of Wounds for the Prevention of Rabies*

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This paper reports on laboratory studies made in guinea-pigs on puncture wounds infected with fixed rabies virus and treated one hour later with various substances, the purpose being to review the experience of previous workers and to explore new approaches to the problem of local treatment of wounds inflicted by rabid animals.

Among the measures affording greater or lesser protection were : nitric acid cauterization; direct application of benzalkonium chloride to the wound or its infiltration, as well as that of methylbenzethonium chloride, proximal to the wound; repeated swabbing and flushing with 20 % soap solution or benzalkonium chloride; local inoculation of procaine anaesthetics; infiltration of the leg wound, or inoculation of the opposite leg, with antirabies gamma-globulin; and infiltration of the wound with interferon prepared in guinea-pig tissue cultures.

Protection was not afforded by flushing with 20 % soap solution alone; topical application of aqueous or tincture preparations of iodine or thiomersal; inoculation of phenoxybenzamine, physostigmine or diphenhydramine hydrochloride; or infiltration of the wound with interferon prepared on monkey kidney tissue cultures.

The authors conclude that, at the present time, very vigorous cleansing of the wound with 20 % soap solution or 2 % benzalkonium chloride, local infiltration of the wound with antirabies serum and—to minimize pain—the use of procaine in saline are indicated in the local treatment of wounds for the prevention of rabies.

The local treatment of wounds made by an animal suspected of being rabid is a vexing problem often faced by physicians and public health authorities. Shaughnessy & Zichis's (1943, 1954) experimental approach to this problem in guinea-pigs was a specially helpful contribution. Reviewing their own data and results obtained by others, Perez Gallardo et al. (1957) have shown the effectiveness of local application to the wound area of nitric acid in preventing rabies infection. The experimental results obtained with nitric acid were not always consistent, however, and additional disadvantages of this time-honoured treatment of wounds are that pain and disfigurement accompany the cauterization of the wounds, and treatment can be applied only to certain areas of the human body. Other methods of treatment less drastic than nitric acid, however, such as mechanical washing and local application of various disinfectants, more frequently gave equivocal results (Perez Gallardo et al., 1957; Shaughnessy & Zichis, 1943, 1954; Ahuja & Suri, 1954, 1955). Deeply penetrating puncture wounds

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presented, and present today, a particularly difficult problem for local treatment. Therefore, this type of puncture wound inflicted on guinea-pigs exposed to rabies virus was the subject of the present study. Various substances either used before or employed for the first time in this study were compared with nitric acid for their effectiveness in preventing rabies infection of the exposed guinea-pigs.

MATERIALS AND METHODS

DESIGN OF EXPERIMENTS

Experiments 1 to 8 (Table 1) were carried out on different days over a period of 10 months.[1] Each worker infected his own group of control guinea-pigs after the animals of a particular group which were to receive treatment had been infected. The guinea-pigs used in this group of experiments weighed between 300 g and 600 g. They were purchased in lots of 100 or more and were randomly distributed in groups of five to a cage. They were then assigned to therapy groups of 10 or 20 guinea-pigs each.

Experiments 1A to 5A (Table 1) were carried out consecutively in a single day.[2] The guinea-pigs used for these experiments were of the Albany standard strain and weighed 300-500 g. They were arranged in groups of 15 by stratified random sampling; the range difference in weight did not exceed 25 g in any one group. Each experiment had its own group of controls (Table 1). Two workers inflicted the wounds and exposed them to rabies virus, and two others applied the treatments.

VIRUS SUSPENSION

This was the CVS strain of fixed rabies virus obtained from the National Institutes of Health, Bethesda, Md., USA. Pool 1 of this virus suspension used in Experiments 1 to 8 and Pool 2 used in Experiments 1A to 5A had end-point titres, respectively, of $10^{6.1}$ LD_{50} and $10^{6.9}$ LD_{50} per 0.03 ml inoculated intracerebrally into 3-4-week-old albino mice (Swiss and Albany standard strain). The pools were kept in individual ampoules of 1 ml each, containing a 20% brain suspension in 50% serum water held frozen at $-60°C$. Before each experiment ampoules were thawed quickly in running water and to each 1 ml of virus suspension were added 4 mg of dried, partially purified, bovine testicular hyaluronidase (Lot M 103 having 691 units/mgm[3]). The hyaluronidase was used to increase the spread of virus from the wound area. In several experiments titrations of the virus suspension used in the experiment itself, but without hyaluronidase, were made at the end of the experimental procedure to determine whether any drop in titre had occurred. No significant drop was noted. The hair on one hind leg was clipped and a small circular area of skin 5-10 mm in diameter was cut away with a small bistoury blade. When bleeding occurred a cotton pledget was applied with pressure. The virus suspension was then applied with a 1-mm-wide platinum loop (1 or 2 loopfuls) to the exposed lateral fascial surface of the gastrocnemius muscle. A Francke lancet, used to obtain drops of blood for haematological study, with acorn tip was then placed on the exposed muscle and a penetrating wound 5 mm deep was made and the lance twirled 10-12 times while in the wound. The wound, and any resulting serum and blood, was left alone for one hour, at which time treatment was applied.

ANAESTHESIA

Pentobarbital sodium solution was injected intraperitoneally in a dose of 12 mg of the drug per pound of body-weight. Complete anaesthesia resulted usually within 5 to 10 minutes. Where required during the course of the experiments, small additional doses were injected.

TREATMENT

All treatment was administered one hour after infection.

Local infiltrations and inoculations

Antirabies gamma-globulin.[4] This was prepared from horse serum and had a protective end-point of

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1: 12 000 when combined with 50 LD_{50} of fixed rabies virus for intracerebral inoculation (0.03 ml) into mice. The gamma-globulin preparation was used in various dilutions (see Table 6), either infiltrated in 0.1-ml quantities beneath the wound for local treatment, or inoculated into the opposite leg to determine the systemic effect. A saline control was inserted in one experiment comprising local infiltration techniques to eliminate the possibility of mere dilution affecting the outcome; no dilution effect was noted. During infiltration of these substances efforts were made to have some of the liquid seep out of the wound canal, but these succeeded only about one-third of the time.

Interferon. This substance was shown to be produced by rabies infection in tissue culture (Kaplan et al., 1960). Two preparations of interferon were used. The interferon used in Experiment 1 (see Table 7) was prepared by infecting guinea-pig kidney monolayers with Newcastle disease virus (NDV) and harvesting the supernate after four days. The supernate was centrifuged for 45 minutes and 1½ hours in two consecutive cycles at 40 000 *g* in a Spinco refrigerated centrifuge to remove the virus. The NDV supernate was tested for interferon action by applying it to fresh guinea-pig kidney monolayers for 24 hours followed by exposure of the cells to western equine encephalitis (WEE) virus. The controls showed cellular degeneration in 24 hours following WEE infection, while the monolayers treated with NDV interferon were protected for two additional days. The interferon used in Experiments 3 and 5 [1] (see Table 7) was prepared in monkey kidney tissue culture and was said to show some activity also in guinea-pig tissue culture systems.

The interferon (0.2 ml) was infiltrated under the wound in the same way as the antirabies gamma-globulin.

Local anaesthetics. The following anaesthetic substances were infiltrated in amounts of 0.5-1.0 ml into an area about 2.5 cm proximal to the wound one hour after infection:

(*a*) Efocaine,[2] a commercial preparation of 1% procaine and 0.25% procaine hydrochloride in a propylene glycol base. In Experiments 5, 6 and 7, and 1A and 5A, 1 mg of the hyaluronidase preparation (see above) was added to each ml of Efocaine.

(*b*) Procaine hydrochloride, 1% in normal saline.

(*c*) Dibucaine hydrochloride (Nupercaine [3]) (*N*, *N*-diethyl-*N'*- (alpha-butoxycinichonyl)-ethylenediamine hydrochloride) 1%, in oil containing 10% benzyl alcohol and 1% phenol.

Quaternary ammonium compounds. Two quaternary ammonium compounds were infiltrated near the wound in the same manner as the anaesthetics one hour after infection:

(*a*) Methylbenzethonium chloride (Hyamine 3500 [4]), *n*-alkyl-dimethyl benzylammonium chloride (50%), a quaternary ammonium preparation, plus water and ethanol (10%).

(*b*) Benzalkonium chloride (Zephirol [5]), also a quaternary ammonium preparation (high molecular alkyl-dimethyl benzylammonium chloride), used in 2% concentration.

Other drugs inoculated. The other drugs inoculated were:

(*a*) Diphenhydramine hydrochloride (Benadryl [6]), an antihistaminic; 25 mg were inoculated subcutaneously, 2.5 cm proximal to the wound, daily for 3 days, followed by 12 mg for an additional 3 days.

(*b*) Phenoxybenzamine (Dibenzyline [3]) (*N*-(phenoxyisopropyl)-*N*-benzyl-chlorethylamine hydrochloride), an adrenergic blocking agent and antihistaminic; 0.2 mg were inoculated subcutaneously, 2.5 cm proximal to the wound, daily for 3 days, and 0.1 mg for 3 further days.

(*c*) Physostigmine (eserine)[7] (0.5% eserine salicylate with methylcellulose), an anticholinesterase; 0.03 ml of this substance was infiltrated once about 2.5 cm proximal to the wound one hour after infection.

Topical applications

Nitric acid (HNO_3, chemical grade, 70% w/w), Lugol's aqueous iodine solution (5%), strong tincture of iodine (7%), 1:1000 aqueous thiomersal,[8] 1:1000 tincture of thiomersal, antirabies gamma-globulin, and 2% benzalkonium chloride were

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[4] Rohm and Haas Co., Philadelphia, Pa., USA.

[5] Farbenfabriken Bayer A.G., Leverkusen, Germany.

[6] Smith, Kline and French, Philadelphia, Pa., USA.

[7] Alcon Laboratories, Inc., Fort Worth, Texas, USA.

[8] Merthiolate, Eli Lilly Co., Indianapolis, Ind., USA.

used for topical application, being applied with a blunt-end probe or a thin cotton swab tightly wound on a stick which was dipped into a vial of the compound and then inserted into the depths of the wound with one full turn. (Use of the substances is shown under " probe " in Tables 2, 3 and 8).

Flushing and swabbing

Two methods were employed.

Flushing only (Experiments 1 and 3, Table 4). The blunt end of a needle attached to a 20-ml syringe was held directly over the wound (Experiment 1), or placed into the wound (Experiment 3). A 20-ml quantity of 20% tincture of green soap followed by 20 ml of water was forcefully ejected from the syringe in a jet into the wound canal.

Swabbing and flushing (Experiments 1A to 5A, Table 3 and Table 4). Both the 2% benzalkonium chloride and 20% soap solutions were applied by a cotton swab inserted to the bottom of the wound and twirled three times, followed by flushing of 5 ml of the fluid as described above. The swabbing and flushing were then repeated a second time.

DIAGNOSIS OF RABIES

Originally, in the early experiments, guinea-pigs were observed for 60 days, but later on the observation period was reduced to 35 days when it was seen that deaths after the 20th day were rare, most of the deaths occurring 6-12 days after inoculation. The inoculated animals were observed daily and the number of sick and paralysed recorded. The paralysis usually occurred first in the inoculated leg, extended rapidly to the opposite leg and became general within 1-2 days. Death occurred usually 2-4 days after the first signs of illness were noted. All dead animals were autopsied and their brain tissue used for preparation of smears for immunofluorescence with antirabies serum (Goldwasser & Kissling, 1959), and for intracerebral subinoculation into mice. The characteristic course of the disease was invariably followed by the demonstration of rabies virus in brain tissue, and therefore in Experiments 1A to 5A clinical diagnosis alone was used as criterion of rabies infection. The brains of all animals dying without showing paralysis were examined by the immunofluorescent technique and subinoculation into mice.

RESULTS

Table 1 gives the mortalities in virus-infected, untreated (control) groups of guinea-pigs used in the 13 separate experiments. Eight of the experiments were individually performed over a 10-month period of time. Five other experiments were all performed as replicate experiments on the same day. The consistency of the challenge technique is shown for all experiments whether performed at one time or over a long period.

Table 2 shows the results of topical HNO_3 treatment either with a metal probe (Experiments 1-7) or with a tightly wound cotton swab on a wooden probe (Experiments 1A-5A). With the former method there was one group out of six in which protection failed (Experiment 5), and two out of five (Experiments 3A and 5A) failed with the second method. On the average, however, HNO_3 exerted a protective effect.

Table 3 shows the results with quaternary ammonium compounds. Three methods of application were used: (*a*) topically with a probe; (*b*) swabbing and flushing; and (*c*) intramuscular inoculation. With topical application with a probe, two groups of animals showed evidence of protection (Experiments 1 and 4) but two groups did not (Experiments 3 and 8). With swabbing and flushing, protection was observed in all five groups, but was greatest in Experiments 3A and 5A. With intramuscular inoculation, protection was evident in both of two experiments.

In Table 4 the effect of flushing with soap solution is shown. With flushing alone (Experiments 1 and 3) failure to protect was shown in both experiments. However, with the more vigorous method of swabbing followed by flushing, both procedures being repeated a second time, the trend to protection was evident in all five experiments in which it was employed (1A to 5A).

Of the local anaesthetics tried (Table 5), the trend of results showed protection with all three.

Table 6 shows the results of three experiments dealing with the use of antirabies gamma-globulin. The results indicate the difficulty of differentiating the local from the systemic effect of the globulin preparation. The undiluted preparation when given either locally or systemically (into the opposite leg) protected, whereas the high dilutions gave little or no protection. The use of undiluted serum topically with a probe gave no protective effect.

TABLE 1
MORTALITY RATIOS OF UNTREATED CONTROL GROUPS (WITH x^2 ANALYSIS)

Experiments	Experiment No.	Mortality in individual experiments		Mortality by groups of experiments		x^2	Degrees of freedom	P value	Interpretation
		Ratio [a]	%	Ratio [a]	%				
Performed on different days over 10-month period	1	6/10	60.0	65/95	68.4	3.99	7	>0.75	No significant difference at 5 % level
	2	7/9	77.7						
	3	15/19	78.9						
	4	13/19	68.4						
	5	6/10	60.0						
	6	8/10	80.0						
	7	5/9	55.6						
	8	5/9	55.6						
All performed on same day	1 A	9/15	60.0	49/75	65.3	3.47	4	>0.25	No significant difference at 5 % level
	2 A	9/15	60.0						
	3 A	8/15	53.3						
	4 A	12/15	80.0						
	5 A	11/15	73.3						
Total	13	114/170							
x^2 analysis for all 13 experiments					67.1	7.65	12	>0.75	No significant difference at 5 % level

[a] No. dead/No. inoculated.

TABLE 2
EFFECT OF NITRIC ACID APPLICATION UPON RABIES-INFECTED WOUNDS OF GUINEA-PIGS

Substance	Method of administration	Experiment No.	Mortality			
			HNO_3-treated		Controls	
			Ratio [a]	%	Ratio [a]	%
HNO_3	Metal probe	1	1/10	10.0	6/10	60.0
		3	4/9	44.4	15/19	79.0
		4	7/16	43.8	13/19	68.4
		5	7/10	70.0	6/10	60.0
		6	2/10	20.0	8/10	80.0
		7	2/8	25.0	5/9	55.5
	Swab probe	1 A	4/15	26.7	9/15	60.0
		2 A	3/15	20.0	9/15	60.0
		3 A	7/15	46.7	8/15	53.3
		4 A	5/15	33.3	12/15	80.0
		5 A	10/15	66.7	11/15	73.3

[a] No. dead/No. inoculated.

TABLE 3

EFFECT OF QUATERNARY AMMONIUM PREPARATIONS UPON RABIES-INFECTED WOUNDS OF GUINEA-PIGS

Detergent	Method of administration	Experiment No.	Mortality			
			Treated		Control	
			Ratio [a]	%	Ratio [a]	%
Benzalkonium chloride, 2 %	Probe	1	1/10	10.0	6/10	60.0
		3	8/10	80.0	15/19	79.0
		4	6/15	40.0	13/19	68.4
		8	8/10	80.0	5/9	55.6
	Swabbing and flushing	1 A	6/15	40.0	9/15	60.0
		2 A	5/15	33.3	9/15	60.0
		3 A	1/15	6.7	8/15	53.3
		4 A	4/15	26.7	12/15	80.0
		5 A	1/14	7.1	11/15	73.3
	Intramuscular inoculation [b]	8	1/10	10.0	5/9	55.6
	Intraperitoneal 2.5 ml	8	5/5 [c]	100.0	5/9	55.6
Methyl-benzethonium chloride, 0.5%	Intramuscular inoculation [b]	8	0/9	0	5/9	55.6

[a] No. dead/No. inoculated.

[b] 1 ml, 2.5 cm proximal to the wound.

[c] Five additional guinea-pigs in this group died within 48 hours of inoculation of the benzalkonium chloride, due to toxicity.

TABLE 4

EFFECT OF SWABBING AND FLUSHING WITH 20% SOAP SOLUTION UPON RABIES-INFECTED WOUNDS OF GUINEA-PIGS

Method employed	Experiment No.	Mortality			
		Treated		Controls	
		Ratio [a]	%	Ratio [a]	%
Flushing only	1	8/10	80.0	6/10	60.0
	3	7/9	77.8	15/19	79.0
Swabbing and flushing	1 A	5/15	33.3	9/15	60.0
	2 A	4/13	30.8	9/15	60.0
	3 A	4/14	28.6	8/15	53.3
	4 A	5/15	33.3	12/15	80.0
	5 A	3/12	25.0	11/15	73.3

[a] No. dead/No. inoculated.

TABLE 5

EFFECT OF LOCAL ANAESTHETICS INJECTED INTRAMUSCULARLY NEAR RABIES-INFECTED WOUNDS OF GUINEA-PIGS

Anaesthetic	Volume injected (ml)	Experiment No.	Mortality			
			Treated		Control	
			Ratio [a]	%	Ratio [a]	%
Efocaine	1.0	5	4/10	40.0	6/10	60.0
		6	2/9	22.2	8/10	80.0
		7	2/8	25.0	5/9	55.6
		8	3/10	30.0	5/9	55.6
	0.5	1 A	7/15	46.7	9/15	60.0
		2 A	3/15	20.0	9/15	60.0
		3 A	8/15	53.3	8/15	53.3
		4 A	8/15	53.3	12/15	80.0
		5 A	7/13	53.8	11/15	73.3
Dibucaine hydrochloride	0.5	1 A	5/15	33.3	9/15	60.0
		2 A	6/15	40.0	9/15	60.0
		5 A	8/15	53.3	11/15	73.3
Procaine	1.0	7	3/8	37.5	5/9	55.5

[a] No. dead/No. inoculated.

TABLE 6

EFFECT OF ANTIRABIES GAMMA-GLOBULIN UPON RABIES-INFECTED WOUNDS OF GUINEA-PIGS

Experiment No.	Mortality ratios [a] of guinea-pigs treated with indicated dilutions of immune serum								Mortality ratios [a] of controls	
	Control		1 : 5		1 : 10		1 : 100	1 : 1 000		
	W [b]	OL [c]	W [b]	OL [c]	W [b]	OL [c]	OL [c]	OL [c]	Untreated	Saline
1	3/10	2/10							6/10	5/10
2	4/9	4/9			6/8	7/10	8/10	8/10	7/9	
3	[6/8 [d]]		4/7	5/7					15/19	

[a] No. dead/No. inoculated.

[b] W = Under the wound.

[c] OL = Injected into opposite leg.

[d] Administered by probe in undiluted form only; other dilutions given intramuscularly.

Table 7 shows the effect of two interferons used. The interferon used in Experiment 1, prepared from guinea-pig tissue, appeared to give some protection, whereas the interferon prepared from monkey tissue used in Experiments 3 and 5 failed to do so.

Table 8 shows results for substances which had no therapeutic effect. These include the local disinfectants iodine and thiomersal, both in aqueous and tincture forms, and various other compounds such as the antihistaminic diphenhydramine, the adrenergic blocking agent phenoxybenzamine, and the cholinergic blocking agent physostigmine.

TABLE 7

EFFECT OF TWO DIFFERENTLY PREPARED INTERFERONS INFILTRATED LOCALLY INTO RABIES-INFECTED WOUNDS OF GUINEA-PIGS

Substance	Method of administration	Experiment No.	Mortality			
			Treated		Control	
			Ratio [a]	%	Ratio [a]	%
Interferon 1 (guinea-pig tissue origin)	Inoculated under wound	1	3/10	30.0	6/10	60.0
Interferon 2 (monkey tissue origin)	Inoculated under wound	3	12/17	70.6	15/19	79.0
		5	7/10	70.0	6/10	60.0

[a] No. dead/No. inoculated.

TABLE 8

SUBSTANCES WHICH HAD NO EFFECT ON RABIES-INFECTED WOUNDS OF GUINEA-PIGS

Substance	Method of administration	Experiment No.	Mortality			
			Treated		Control	
			Ratio [a]	%	Ratio [a]	%
Iodine	Probe	3 [b]	9/10	90.0	15/19	78.9
		3	10/18	55.6	13/19	68.4
		1 A	11/15	73.3	9/15	60.0
		2 A	8/15	53.3	9/15	60.0
		5 A	10/15	66.7	11/15	73.3
Thiomersal	Probe	3 [c]	6/9	66.7	15/19	78.9
		4	13/18	72.2	13/19	68.4
Diphenhydramine	Intramuscular inoculation [d]	1	6/8	75.0	6/10	60.0
Phenoxybenzamine	Intramuscular inoculation [d]	5	7/10	70.0	6/10	60.0
Physostigmine	Intramuscular inoculation [d]	8	4/9	44.4	5/9	55.6

[a] No. dead/No. inoculated.
[b] 5% Lugol's solution; all other experiments in this group were with a 7% tincture preparation.
[c] Aqueous 1:1 000; the other experiment (No. 4) was performed with tincture 1:1 000.
[d] See "Material and Methods" for dosage.

DISCUSSION

The experimental method described above was devised to provide a uniform testing mechanism for studying therapeutic agents in the treatment of wounds to prevent the spread of rabies virus from the site of exposure to the central nervous system. A puncture wound was selected as representing the most difficult type encountered in practice. It would seem from a comparison of results obtained over a 10-month period with the results of five replicate experiments performed on the same day that a

consistent challenge procedure had been devised. Against this consistent challenge the effects of various treatments must be examined from two points of view: (1) the ability to produce a significant protective effect, and (2) the ability to produce this effect consistently.

The results of treatment with nitric acid corroborate those obtained by others as to the protective efficacy of this compound applied directly to the wound. However, as already shown by Perez Gallardo et al. (1957), the results were not very consistent in repeated experiments. This inconsistency is undoubtedly caused by variables in the method of treatment with the acid as well as in the nature of the inflicted wounds. These variables, if present in the laboratory, will certainly be present in the course of normal medical practice throughout the world. The results obtained above, coupled with the caustic properties of nitric acid, suggest great limitations in the usefulness of this chemical in wound therapy for rabies prevention.

A similar beneficial effect was noted with flushing of the wound. Repeated swabbing and flushing with 20% green soap or 2% benzalkonium chloride did exert a protective effect,[1] although in some individual experiments the results were equivocal. However, repeated flushing, with copious amounts of 20% green soap and water but without swabbing, failed to show any protective effect in two experiments. This again places an emphasis on the method of application rather than on the nature of the compound. It is noteworthy that even with the vigorous mechanical procedures protection was far from complete. A range extending from complete lack of protection to significant but not complete protection by the use of soap or detergent solutions has been observed by other workers (Ahuja & Suri, 1954, 1955; Shaughnessy & Zichis, 1943, 1954; Perez Gallardo et al., 1957).

The action of 2% benzalkonium chloride in the present experiments, as well as in those of previous workers using a 1% concentration (Shaughnessy & Zichis, 1943, 1954) is of considerable interest. The question arises whether this action was virucidal in nature, a mechanical cleansing one, systemic, or a combination of these. Until now it has been assumed to be principally a cleansing and/or specifically virucidal action. It will be noted that in two experiments (Experiments 1 and 3, Table 3) topical application of the substance with a probe—and in Shaughnessy & Zichis' experiments (1943, 1954) with a brush—was sufficient to protect a significant number of animals. When, however, 2% benzalkonium chloride was inoculated 2.5 cm proximal to the wound, a high order of protection occurred (Table 3) as compared with a control group inoculated intraperitoneally. This specific local action has been confirmed also in mice by Cohen et al.[2] It is therefore difficult to differentiate the mechanical effectiveness of swabbing and flushing with 2% benzalkonium chloride from the possible pharmacological action which could have resulted from diffusion of the compound into the surrounding tissue. A protective effect was also observed with another quaternary ammonium compound, methylbenzethonium chloride, inoculated proximal to the wound (Table 3). It should be noted that in general the quaternary ammonium compounds, in addition to being cationic detergents, may act as nerve-blocking agents (Goodman & Gilman, 1955).

In this connexion, the protective effect of the various preparations of procaine is noteworthy (Table 5).[3] How their blocking action affects the centripetal progress of the infective component of the rabies virus remains to be determined, especially when the time element of action is limited to a few hours. It has been shown that rabies virus inoculated intramuscularly can persist at the site of inoculation for 24 hours (Schindler, 1961a), and in some instances up to 72 hours (Habel, 1954), but the virus level drops sharply shortly after inoculation (Schindler, 1961a). One of the major reasons, therefore, for the effectiveness of the local anaesthetics and related compounds may be simply that the rabies virus is held locally in abeyance until a die-off occurs to levels below the minimum infective dose.

Attention should also be paid to the greater uniformity of results obtained when various products (procaine and derivatives and quaternary ammonium compounds) were introduced parenterally rather than by probe or by swabbing and flushing (Tables 3, 4 and 5). Although the level of protection with these compounds when inoculated is not quite as great as might be desired, a consistent beneficial effect has been demonstrated. The use of local anaesthetics by practitioners to relieve the pain of local wounds should be encouraged also for its

[1] It should be noted that soap and quaternary ammonium compounds are chemically incompatible.

[2] See the note on page 831 of this issue.

[3] In their cationic form local anaesthetics in general —but not as used in these experiments—are quaternary ammonium derivatives (Goodman & Gilman, 1955).

possible specific beneficial effects in antirabies therapy.

In our present experiments the specific local action of antirabies gamma-globulin infiltrated under the wound could not be differentiated from its systemic action (Table 7). When the undiluted globulin preparation was applied with a probe into the wound canal it was apparently insufficient to protect. Our experiments, however, and those of others all suggest the usefulness of local infiltration, particularly if performed soon after infection (Habel, 1954; Ercegovac, 1956; Perez Gallardo et al., 1957; Schindler, 1961b; Soloviev & Kobrinski;[1] and unpublished observations by Kaplan & Paccaud). Because of the observed persistence of rabies virus at the site of intramuscular inoculation for 24 hours and even up to 72 hours (Habel, 1954; Schindler, 1961a) local infiltration with antirabies serum should not be excluded as a possible preventive measure at any time within such a period following exposure. (The use of serum locally should not be confused with or replace its systemic use as recommended by the WHO Expert Committee on Rabies (1960)).

The interferon prepared from guinea-pig tissue in one experiment gave some indication of protection, while the interferon prepared from monkey tissue did not (Table 7). This may be due to the greater specificity of the guinea-pig interferon when used in a homologous species, as has been indicated by other work with interferon (Tyrrell, 1959). Further work, however, is needed to clarify this point in connexion with rabies protection.

Various products failed to offer any protection in our studies. Aqueous and tincture preparations of iodine and thiomersal were ineffective, as were phenoxybenzamine, physostigmine and diphenhydramine (Table 8).

Diphenhydramine is an antihistaminic. It is of interest that large doses of histamine dihydrochloride and water-soluble histamine derivatives have been shown to decrease the mortality from experimental rabies infection in mice when it is injected intramuscularly at the same place as the virus (Schindler, 1960, and personal communication). This action was still evident when the histamine was injected 16 hours after infection. Histamine appeared to have little or no effect on the rabies virus itself *in vitro* (Schindler, 1960). The guinea-pigs inoculated with diphenhydramine in our Experiment 1 died of rabies several days sooner than other guinea-pigs in the same experiment, and this may reflect a partial neutralization of the histamine-inhibiting effect on rabies infection described by Schindler. Earlier deaths in animals were not observed in the group receiving phenoxybenzamine, also an antihistaminic.

The belief that alteration of nerve function could somehow be involved in the mechanism of blockage of the centripetal spread of rabies virus led us to the testing of an adrenergic blocking agent, phenoxybenzamine, and a cholinergic blocking agent, physostigmine. Initial work with these products indicated no beneficial effect. As stated previously, a protective effect was obtained with the procaine derivatives and quaternary ammonium compounds inoculated near the site of the wound.

Thus, for a more complete understanding of the pathogenesis of rabies further investigations are required to determine the mechanism of pharmacological agents acting on the central and peripheral nervous systems, and, in connexion with the pathogenesis of rabies, to determine the importance of the physical volume and the character of the inoculum in affecting centripetal transmission of the rabies virus along peripheral nerves.

It would follow that the rationale for therapy suggested by these guinea-pigs studies would be:

1. To swab and flush the wound vigorously and repeatedly with a soap *or* quaternary ammonium solution (preferably benzalkonium chloride pending further work on other such compounds).

2. In order to minimize pain, to infiltrate the wound area, especially proximal to the wound, with a local anaesthetic such as procaine with avoidance of further trauma of the wound. Procaine in saline solution would be the anaesthetic of choice, representing the most universally available and simplest of these compounds.

3. To infiltrate under the wound itself with hyperimmune serum where indicated.

4. To proceed with the recommended systemic applications of hyperimmune serum and vaccine as recommended by the WHO Expert Committee on Rabies (1960).

[1] See the article on page 777 of this issue.

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RÉSUMÉ

Poursuivant les recherches sur le traitement local des blessures dues à la morsure d'animaux enragés ou soupçonnés de l'être, les auteurs ont repris quelques essais faits par d'autres chercheurs, et ont mis à l'épreuve certains procédés nouveaux, afin d'enrayer la propagation du virus vers les centres nerveux.

Les essais ont été effectués sur des cobayes, blessés expérimentalement et infectés localement par du virus fixe, puis traités une heure après infection par divers procédés. Au nombre de ceux qui ont donné des résultats satisfaisants figurent:

L'application d'acide nitrique par sonde ou écouvillon directement dans la blessure; l'application de composés d'ammonium quaternaire, tels que le chlorure de benzalkonium, directement dans la blessure, ou par injection, à 2,5 cm de celle-ci; le lavage énergique et l'écouvillonage avec une solution savonneuse à 20% constamment renouvelée, et une solution de chlorure de benzalkonium à 2%; l'injection d'anesthésiants, tels que la procaïne, l'Efocaïne, le chlorhydrate de dibucaïne, qui sont chimiquement apparentés aux composés d'ammonium quaternaire; l'infiltration locale sous la blessure de gammaglobuline antirabique (qui, toutefois, ne donna pas des résultats supérieurs à ceux qu'assurait l'inoculation de dilutions analogues dans le membre opposé); l'infiltration dans la blessure d'interféron préparé sur des cultures de tissu rénal de cobaye.

En revanche, certains procédés ou certaines techniques d'application ont abouti à un échec. Ce sont: le lavage abondant de la blessure avec une solution de savon à 20% (sans adjuvant); l'application locale d'iode ou de thiomersal; l'injection à 2,5 cm de la plaie, d'agents neuroplégiques, tels que la phénoxybenzamine et la physostigmine, et un antihistaminique, tel que le chlorhydrate de diphénhydramine; l'infiltration d'interféron préparé sur tissu rénal de singe.

Les indications pratiques que l'on peut tirer des essais effectués jusqu'ici sont les suivantes: Nettoyer, et laver énergiquement la plaie avec une solution savonneuse à 20% *et* une solution d'un composé de l'ammonium quaternaire (le chlorure de benzalkonium, au stade actuel des recherches). Infiltrer dans la zone proximale de la blessure un anesthésique local, en évitant d'aggraver la blessure; la procaïne en soluté salin est actuellement le plus simple et le plus répandu de ces produits. Infiltrer sous la blessure, s'il y a lieu, du sérum hyperimmun. Ensuite, appliquer la séro- et la vaccinothérapie, selon les recommandations du Comité OMS d'experts de la Rage.

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