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Studies on the Local Treatment of Rabies-infected Wounds

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Local treatment of wounds infected with rabies virus is a valuable adjunct to the use of antiserum, vaccine or both in preventing disease. In the absence of effective chemotherapeutic agents for systemic use, improved treatment awaits a better understanding of the fate of virus at the site of exposure and of the method of spread to the central nervous system.

The studies on experimental animals reported in this paper serve further to evaluate methodology in treatment; to evaluate the effectiveness of various substances, including antirabies serum, soap and benzalkonium chloride; and to explore the possible role of blocking agents in preventing rabies. The results obtained provide further evidence of the importance of first-aid procedures. Certain oil-based local anaesthetics, almond oil, benzyl alcohol and benzalkonium chloride, interfere with motor function and exert a marked sparing effect when inoculated intramuscularly above the site of infection; aqueous or saline-based local anaesthetics and saline do not interfere and fail to protect. The role of blocking agents, if any, in preventing rabies in man is unknown.

Prompt and adequate treatment of wounds infected with rabies virus is important in the prevention of disease. Our studies of local wound therapy have indicated dramatically the effectiveness of simple first-aid procedures such as scrubbing and flushing with tap water, soap and water, or benzalkonium chloride in the treatment of experimentally infected non-puncture wounds and confirm the sparing effect of certain local anaesthetics noted by Kaplan et al. (1962). Such anaesthetics exert a local rather than a systemic action with the sparing effect apparently due to a blocking action rather than to a specific virucidal effect. A relationship was observed between loss of motor function and resistance to infection. Aqueous preparations which do not noticeably interfere with motor function tend to give little or no protection; oil-base local anaesthetics or substances such as benzalkonium chloride give marked protection. The use of antirabies serum, benzalkonium chloride, soap and other agents in the treatment of deep puncture wounds was further explored.

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MATERIALS AND METHODS

All experiments were carried out between 12 September 1961 and 13 July 1962. Guinea-pigs and mice were of the Albany Hartley and Albany standard strains, respectively, and were selected by stratified random sampling according to weight, and, if both males and females were used, according to sex as well. Unless otherwise specified, guinea-pigs weighed 300-400 g and mice 20-24 g. Mice used for virus infectivity titrations weighed 10-12 g.

The source of virus was 20% suspensions of infective mouse brain, prepared from the control virus standard (CVS) strain of fixed rabies virus obtained from the National Institutes of Health. Sodium chloride solution (0.85%) containing 2% normal horse serum was used as the diluent in all experiments. Virus preparations were stored at −56°C until used. Their LD_{50} values ranged from 0.03 ($10^{-7.5}$) to 0.03 ($10^{-6.6}$) ml when inoculated intracerebrally in mice. Virus suspensions were titrated for infectivity before and after each experiment; no appreciable drop in titre was noted.

Unless otherwise specified, guinea-pigs were infected according to the method of Kaplan et al. (1962) except that the depth of the wounds was 6 mm. Superficial wounds were made by removing two circular areas of skin 5-10 mm in diameter in the cervical region. Virus was applied to each lesion as in the other experiments. Mice were infected by inoculating 0.03 ml of a 10% suspension of infective mouse brain into a hind leg; 0.015 ml was injected subcutaneously, the remainder intramuscularly. One worker infected all animals; not more than two workers applied treatment. In all but one experiment the treated and control animals, 8-20 per group, were further divided into subgroups of five animals or less. To avoid bias and to ensure greater uniformity of infection and treatment, subgroups of treated and control animals were processed alternately. A 5-ml syringe with blunted 22-gauge (0.70-mm) needle, held at the orifice or inserted to the bottom of the wound, was used in flushing. Rabies antiserum for the experiments reported in Tables 2 and 3 contained approximately 413 units per ml; that for other studies had 100 units per ml.

Local anaesthetics and other substances used in four or five sites as blocking agents were injected intramuscularly approximately 0.5 cm and 1.0-1.5 cm proximal to the wound in mice and guinea-pigs, respectively; the dose was 0.5 ml in guinea-pigs, 0.045 ml or 0.09 ml in mice. In certain experiments guinea-pigs were injected in the region of the sciatic notch between the trochanter of the femur and the vertebral column as described by Schakell (1935). Unless otherwise specified, animals were treated one hour after infection. Test animals were observed at least daily for 30 days, only those dying of rabies being considered positive. Signs of rabies customarily appeared between the 5th and the 12th day in guinea-pigs and the 6th and 10th day in mice. Those animals dying without having previously shown signs of rabies were examined by the fluorescent antibody technique, by animal inoculation, or by both methods. Any animal negative for rabies by these tests was eliminated from the experiment, although this was rarely necessary.

Dibucaine hydrochloride (Nupercaine—*N*, *N*-diethyl-*N'* (alpha-butoxycinchoninyl)-ethylenediamine hydrochloride) was obtained from Ciba Pharmaceutical Products, Inc., Summit, N.J.; tetracaine hydrochloride (Pontocaine — dimethylamino-ethyl-*p*-*N*-butylaminobenzoate) from Winthrop Laboratories, New York, N.Y.; cyclomethycaine hydrochloride (Surfacaine—3-(2-methylpiperidino) propyl *p*-cyclohexyloxybenzoate) from Eli Lilly Co., Indianapolis, Ind.; dimethisoquin (Quotane—3-butyl-1-(dimethylaminoethoxy)isoquinoline) from Smith, Kline and French Laboratories, Philadelphia, Pa.; propylene glycol (1,2-propanediol) from Eastman Kodak Company, Rochester, N.Y.; benzalkonium chloride (Zephiran—high molecular alkyl-dimethyl benzylammonium chloride) from Winthrop Laboratories, New York, N.Y.; and rabies antiserum from Lederle Laboratories, Pearl River, N.Y.

The statistical significance of observed differences in proportions reacting was tested in accord with what Fisher (1934) has called " the exact treatment of two-by-two tables ". This is based upon a critical probability P that Thompson (1934) had expressed in terms of his four-variable ψ-function, later supplying convenient tables (1955) and various methods of computing P, including the use of a rapid, precise electronic computer programme.[1] The difference in reacting proportions is statistically significant if $P \leq 0.025$ and highly significant if $P \leq 0.005$.

EXPERIMENTS

The effectiveness of treating deep-puncture wounds infected with rabies virus by swabbing with saline, rabies antiserum and 2% aqueous benzalkonium chloride, either singly or in combination, was first studied. Each wound was treated with a total of six cotton-tipped applicators, saturated with the agent used for treatment, immersed to the depth of the wound, twisted four times, and withdrawn. When combinations of benzalkonium chloride and saline, benzalkonium chloride and serum, or serum and saline were used, each animal was treated with three applicators for each agent in turn; two of each were used for the combined benzalkonium chloride, serum and saline treatment. The greatest protection was apparently obtained in animals treated with antiserum alone or antiserum combined with either saline, benzalkonium chloride or both (Table 1). The differences from the controls were highly significant statistically for all treatments excepting that with saline alone, which seemed virtually without effect, suggesting that simple swabbing is not adequate treatment for deep wounds. Though there were some differences between the proportions surviving with the various treatments, such differences were not statistically significant except with saline alone, where the survival pro-

[1] Thompson, W. R. *Precise electronic computation of Ψ (r,s,u,v), its exact tabulation and "exact treatment" of two-by-two tables* (unpublished).

TABLE 1
COMPARATIVE EFFICACY OF RABIES ANTISERUM, BENZALKONIUM CHLORIDE AND SALINE IN SWAB TREATMENT OF DEEP PUNCTURE WOUNDS IN GUINEA-PIGS

Treatment substance [a]	No. reacting [b]	% reacting
Antirabies serum	3/20 (HS)	15.0
Saline and serum	3/20 (HS)	15.0
Saline	16/20	80.0
Benzalkonium chloride	6/20 (HS)	30.0
Benzalkonium chloride and saline	8/20 (HS)	40.0
Benzalkonium chloride and serum	5/20 (HS)	25.0
Benzalkonium chloride, serum and saline	5/20 (HS)	25.0
Controls	18/20	90.0

[a] Treatment was given approximately one hour after infection.
[b] Results expressed as the number of animals reacting over the number inoculated. HS indicates that the difference from the controls is statistically highly significant.

portion was highly significantly less than for the other treatments.

The efficacy of rabies antiserum administered by various methods was next studied (Table 2). Excellent protection was obtained when antiserum was administered locally in amounts of less than 41.3-82.6 International Units by either deep flushing, a combination of swabbing and deep flushing, or swabbing plus infiltration of the musculature surrounding the wound. Highly significant but apparently less protection also resulted from swabbing alone. Apparently little or no protection resulted when antiserum was used in the opposite leg (inoculation or swabbing) or from superficial flushing of the infected wound; in these instances the results did not differ significantly from those for the controls.

The effect of varying the time interval between infection and treatment in both guinea-pigs and mice was investigated. With the former, groups of 20 animals each were infected and the non-control animals subsequently treated by swabbing the wounds with six applicators saturated with hyperimmune serum 1, 3, 6, 12, 24, 48, and in one experiment, 72 hours after infection.

In the first experiment each guinea-pig received not more than 41.3 International Units of antiserum administered by swabs as in the previous experiments. Although there seemed to be some evidence of protection in the groups treated not later than 12 hours after infection, the results were not statistically significantly different from those for the controls; and the latter showed no difference from results of treatment 24 or more hours after infection (Table 3). The mortality in the controls was only 40%.

Groups of guinea-pigs were used to study the comparative effectiveness of treating wounds by a combination of swabbing and flushing with either rabies antiserum or 2% aqueous benzalkonium chloride. Each animal treated with antiserum received approximately 82.6 International Units, the wound being swabbed consecutively on each of two occasions with two applicators saturated with antiserum followed by deep flushing with 1.0 ml of diluted antiserum. The results (Experiment 2, Table 3) indicate that both antiserum and benzalkonium chloride significantly protected guinea-pigs when used within 6 and 12 hours respectively following infection. Benzalkonium chloride equalled or out-performed antiserum at all times; there was suggestive evidence of protection in animals treated as long as 24 and 48 hours following infection but the results were not significantly different statistically from those for the controls. Interpretation of

TABLE 2
COMPARATIVE EFFICACY OF DIFFERENT METHODS OF TREATMENT WITH RABIES ANTISERUM IN DEEP PUNCTURE WOUNDS IN GUINEA-PIGS

Treatment method [a]	Units of antiserum	No. reacting [b]	% reacting
Swab	<41.3	5/20 (HS)	25.0
Swab (opposite leg)	<41.3	17/19	89.5
External flushing	41.3	16/20	80.0
Deep flushing	41.3	1/19 (HS)	5.3
Flushing and swabbing	<82.6	0/18 (HS)	0.0
Swab + intramuscular infiltration	<82.6	1/19 (HS)	5.3
Intramuscular injection (opposite leg)	82.6	12/20	60.0
Controls	—	16/19	84.2

[a] Treatment was given approximately one hour after infection.
[b] Results expressed as the number of animals reacting over the number inoculated. HS indicates that the difference from the controls is statistically highly significant.

TABLE 3
EFFECT OF EARLY TREATMENT IN PROTECTING GUINEA-PIGS AND MICE AGAINST RABIES

Interval between infection and treatment (hours)	Experiment 1 [a] (guinea-pigs)	Experiment 2 [a] (guinea-pigs)		Experiment 3 [a] (mice)	
		Swabbing and flushing with:		Intramuscular inoculation:	
	Swab treatment with antiserum	Antiserum	2% aq. benzalkonium chloride	Dibucaine hydrochloride vehicle	1% aq. benzalkonium chloride
1	6/20	3/20 (HS)	0/20 (HS)	3/20 (HS)	2/20 (HS)
3	2/20	4/20 (HS)	1/16 (HS)	4/10 (HS)	1/10 (HS)
6	5/20	4/20 (HS)	4/20 (HS)	8/10	6/10
12	6/19	11/20	5/19 (HS)	10/10	8/10
24	8/20	15/20	11/18	10/10	10/10
48	8/20	16/19	12/18	9/10	9/10
72	8/20				
Controls reacting:					
No.	8/20	30/39		19/20	
%	40.0	76.9		95.0	

[a] Results expressed as the number of animals reacting over the number inoculated. HS indicates that the difference from the controls is statistically highly significant.

the data in those animals given benzalkonium chloride was complicated by the fact that local swellings resulting from treatment were observed in practically every animal. It is not evident whether the marked beneficial effect observed in such animals is due to the specific virucidal action of benzalkonium chloride, to a cleansing or traumatic effect induced by the agent, or to both.

Experiments with guinea-pigs require large numbers of expensive animals to counterbalance the influence of differences in individual susceptibility to rabies virus. Mice may or may not be more uniformly susceptible; they are, however, widely available and comparatively inexpensive and allow of evaluation in a different species. They have been used successfully to evaluate the efficacy of benzalkonium chloride and other substances in local wound treatment in this laboratory since 1958 and by Cohen et al. (1962). It was determined by trial and error that the best results were obtained with mice inoculated in the region of the gastrocnemius muscle by the combined subcutaneous and intramuscular routes with 20 to 30 million LD_{50} of CVS virus as determined by intracerebral titration. This dose consistently induced a mortality of 98%-100% in untreated animals.

The effects of 1% aqueous benzalkonium chloride and of the dibucaine hydrochloride vehicle[1] were investigated in mice inoculated intramuscularly approximately 0.5 cm proximal to the rabies-infected wound with 0.045 ml of either substance. The groups of 10-20 animals each were treated 1, 3, 6, 12, 24, and 48 hours, respectively, after infection. The results (Experiment 3, Table 3) indicate that both the dibucaine hydrochloride vehicle and benzalkonium chloride exerted a marked protective effect in those animals treated 1 or 3 hours after infection. The mortality increased markedly when treatment was delayed more than 3 hours; there was no statistically significant evidence of protection. The challenge was severe in that 95% of the control animals died.

Many purported exposures to rabies are dubious or consist of bruises, abrasions, lacerations and other minor wounds, and the effectiveness of simple first-aid procedures in preventing disease in experimentally infected guinea-pigs was therefore explored next in four studies, using groups of five animals each with non-puncture cervical wounds. Each wound was infected with approximately one million LD_{50}

[1] The vehicle includes almond oil, 10% benzyl alcohol and 1% phenol.

TABLE 4
USE OF FIRST-AID PROCEDURES IN TREATING SUPERFICIAL WOUNDS THREE HOURS AFTER INFECTION

Treatment substance	Experiment 1 [a]	Experiment 2 [a]	Experiment 3 [a]	Experiment 4 [a]	Total	
					No. reacting [a]	% reacting
Tap water	1/5 (S)	0/5 (HS)	0/4 (S)	0/5	1/19 (HS)	5.3
20% soap	0/5 (HS)	1/4	1/5 (S)	0/5	2/19 (HS)	10.5
Ivory soap	1/5 (S)	1/5 (S)	0/5 (HS)	0/5	2/20 (HS)	10.0
Benzalkonium chloride (1%)	1/5 (S)	0/5 (HS)	1/5 (S)	0/5	2/20 (HS)	10.0
Ivory soap and serum	1/5 (S)	0/5 (HS)	1/5 (S)	0/5	2/20 (HS)	10.0
Controls	5/5	5/5	5/5	3/5	18/20	90.0

[a] Results expressed as the number of animals reacting over the number inoculated. S indicates that the difference from the controls is statistically significant; HS that it is highly so.

of CVS virus as determined by intracerebral titration in mice and treated 3 hours after infection by scrubbing and flushing the wounds with cotton pledgets impregnated with warm tap water, 20% soap solution, 1% aqueous benzalkonium chloride, or Ivory soap and water both with and without the addition of topically applied rabies antiserum. Despite severe challenge, as manifested by the 90% mortality in the control animals, almost complete protection was observed in all groups irrespective of treatment. Not more than two treated animals in each treated group died of rabies (Table 4), and differences from controls are highly significant statistically.

Following the observation by Kaplan et al. (1962) that local anaesthetics such as dibucaine hydrochloride in oil, Efocaine and procaine hydrochloride protect against rabies, efforts were made to determine the method by which protection was induced and what drugs were most effective. Three experiments were made with guinea-pigs. In the first (Table 5), dibucaine hydrochloride in oil and certain of its components—dibucaine hydrochloride vehicle, almond oil and 10% benzyl alcohol—were tested for effectiveness in preventing rabies when inoculated intramuscularly proximally to infected puncture wounds. The remaining component, 1% phenol, was not tested.

The results again indicate the effectiveness of dibucaine hydrochloride in oil in preventing rabies and were highly significantly different from those for the controls. The dibucaine hydrochloride vehicle also appeared significantly effective. Two of the components—almond oil and benzyl alcohol—and dibucaine hydrochloride in oil swabbed in the rabies-infected wound yielded somewhat greater survival proportions than the controls but these were not statistically significant. Since this drug had little or no effect when injected intramuscularly in the opposite leg, protection is apparently due to local rather than to systemic action.

In the second experiment the effectiveness of four local anaesthetics—dibucaine hydrochloride in oil, dibucaine hydrochloride in saline, propylene glycol and tetracaine hydrochloride—was tested. Propylene glycol gave significant indication of protection; dibucaine hydrochloride in oil seemed only slightly less effective. Tetracaine hydrochloride showed some evidence of protection, while dibucaine hydrochloride in saline did not. In a third experiment (Table 5) the survival proportions obtained with dibucaine hydrochloride in oil, propylene glycol, dimethisoquin and cyclomethycaine hydrochloride were greater than in the controls, but the differences were not statistically significant. There appeared to be little or no protection with dibucaine hydrochloride in saline or tetracaine hydrochloride. The low mortality in the control animals in this experiment (42.8%) was attributed to the use of guinea-pigs weighing 400-450 g, and may have been largely responsible for the failure to obtain significant differences.

A fourth experiment (Table 5), this in mice, again confirmed the protective activity of the dibucaine hydrochloride vehicle and 1% aqueous benzalkonium chloride when injected proximally to the

TABLE 5

USE OF LOCAL ANAESTHETICS AND OTHER SUBSTANCES IN TREATMENT OF RABIES-INFECTED ANIMALS

Treatment [a]			Experiment 1 [b] (guinea-pigs)	Experiment 2 [b] (guinea-pigs)	Experiment 3 [b] (guinea-pigs)	Experiment 4 [b] (mice)
Substance	Method [c]	Site				
Dibucaine hydrochloride (oil)	Swab	Same leg	7/20			
Dibucaine hydrochloride (oil)	I.M.	Same leg	2/20 (HS)	11/20	1/13	
Dibucaine hydrochloride (oil)	I.M.	Opp. leg	11/20			
Dibucaine hydrochloride (saline)	I.M.	Same leg		16/20	5/13	
Dibucaine hydrochloride vehicle	I.M.	Opp. leg				20/20
Dibucaine hydrochloride vehicle	I.M.	Same leg	4/20 (S)			3/20 (HS)
Almond oil	I.M.	Same leg	7/20			
10% benzyl alcohol	I.M.	Same leg	7/20			
Propylene glycol	I.M.	Same leg		10/20 (S)	3/15	
Tetracaine hydrochloride	I.M.	Same leg		13/20	8/15	
Dimethisoquin	I.M.	Same leg			3/15	
Cyclomethycaine hydrochloride	I.M.	Same leg			2/13	
Saline	I.M.	Same leg				20/20
1% aqueous benzalkonium choride	I.M.	Same leg				2/20 (HS)
1% aqueous benzalkonium chloride	I.M.	Opp. leg				20/20
Controls reacting:						
No.			11/18	17/20	6/14	19/20
%			61.1	85.0	42.9	95.0

[a] Treatment was given approximately one hour after infection.

[b] Results are expressed as the number of animals reacting over the number inoculated. S indicates that the difference from the controls is statistically significant; HS that it is highly so.

[c] I.M. = intramuscular injection.

wound, and showed no evidence of protection when saline was similarly injected and when either the dibucaine hydrochloride vehicle or benzalkonium chloride was inoculated into the opposite leg.

Since the effectiveness of local anaesthetics in preventing rabies might be due, at least in part, to a local or nerve-blocking action, six local anaesthetics, 1% aqueous benzalkonium, the dibucaine hydrochloride vehicle and rabies antiserum were tested in mice for their ability to prevent rabies when inoculated intramuscularly above the site of virus injection. This experiment included the use of four local anaesthetics in aqueous or saline solution (tetracaine hydrochloride, dibucaine hydrochloride, dimethisoquin and cyclomethycaine hydrochloride) and also dibucaine hydrochloride and propylene glycol. In the case of each agent, 10 mice were inoculated intramuscularly with a 0.045-ml amount and 10 with 0.09 ml one hour after virus inoculation. Mice were examined for loss of motor function in the treated extremity at 6 and 24 hours and 5 days after inoculation of the agent. Signs of impaired function, principally curling of the toes and complete or partial paralysis of the inoculated extremity, usually appeared almost immediately after inoculation and, apparently depending upon the extent of involvement, persisted in some cases for a week or more. Such reactions (Table 6) were most noticeable in animals inoculated with benzalkonium chloride and the oil-base preparations. This was highly significantly different from the results in the controls in which paralysis was not observed. Paralysis was not found in those animals given antiserum and seldom in those given the other aqueous or saline preparations. There was an apparent relationship between loss of motor function and resistance to infection. Aqueous or saline solutions of tetracaine hydrochloride, dibucaine

TABLE 6

LOSS OF MOTOR FUNCTION IN THE INOCULATED EXTREMITY IN RELATION TO THE INCIDENCE OF RABIES IN MICE

Treatment substance [a]	No. reacting [b] at dose:		Percentage surviving [c]	Percentage with paralysis in 6 hours [c]	Loss of motor function [c]			
	0.045 ml	0.09 ml			Degree	Time after inoculation: 6 hrs	24 hrs	5 days
Dibucaine hydrochloride (oil)	2/10 (HS)	1/10 (HS)	90	100	Complete	10 (HS)	4	1
					Partial	0	6	5
Dibucaine hydrochloride vehicle	6/10	0/10 (HS)	100	100	Complete	9 (HS)	4	0
					Partial	1	5	10
Dibucaine hydrochloride (saline)	8/9	4/4	0	0	Complete	0	0	0
					Partial	0	0	0
1% aqueous benzalkonium chloride	1/9 (HS)	— [d]	89 [c]	100 [c]	Complete	10 (HS)	9	3
					Partial	0	1	5
Propylene glycol	4/10 (S)	5/10 (S)	50	70	Complete	3	0	0
					Partial	4	2	7
Dimethisoquin	10/10	8/10	20	20	Complete	1	0	0
					Partial	1	1	0
Tetracaine hydrochloride	10/10	10/10	0	0	Complete	0	0	0
					Partial	0	0	0
Cyclomethycaine hydrochloride	8/10	8/10	20	0	Complete	0	0	0
					Partial	0	0	0
Antiserum	7/10	5/10 (S)	(50)	(0)	Complete	0	0	0
					Partial	0	0	0
Controls	10/10	10/10	0	0	Complete	0	0	0
					Partial	0	0	0

[a] Treatment was given approximately one hour after infection.

[b] Results are expressed as the number of animals reacting over the number inoculated. S indicates that the difference from the controls is statistically significant; HS that it is highly so.

[c] Measured in mice inoculated with 0.09 ml, except for those given benzalkonium chloride, for which calculations are based on the 0.045-ml inoculation.

[d] All mice were dead from benzalkonium treatment at 2 days.

hydrochloride, cyclomethycaine hydrochloride and dimethisoquin offered little or no evidence of protection; whereas benzalkonium chloride, dibucaine hydrochloride in oil or its vehicle and propylene glycol yielded significantly greater survival proportions than the controls, the differences being, in fact, highly significant except for the propylene glycol. Benzalkonium chloride proved toxic for mice; all 10 inoculated with 0.09 ml and one of 10 given 0.045 ml died within the following two days. Rabies antiserum again appeared of value in preventing rabies. In a similar experiment in guinea-pigs, using chillies (*Capsicum annuum* var. *accuminatum*) prepared according to the method of Negrete et al. (1957), there was no protection when 0.5 ml was inoculated intramuscularly above the site of infection; mortality in both treated and control groups was 17/20.

Tests for the virucidal effect of dibucaine hydrochloride in saline, dibucaine hydrochloride in oil, the dibucaine hydrochloride vehicle, propylene glycol, benzalkonium chloride, and soap were also made by inoculation of mice with previously incubated mixtures of agent and virus (Table 7). Four doses in a succession of fivefold dilutions of each substance were combined with approximately

TABLE 7
TESTS FOR VIRUCIDAL ACTIVITY OF DIFFERENT SUBSTANCES BY RESPONSE IN MICE

Test substance	Concentration of test substance [a]							
	1/1 000	1/5 000	1/25 000	1/125 000	1/5	1/25	1/125	1/625
Dibucaine hydrochloride in saline	7/7	8/8	8/8	8/8				
Dibucaine hydrochloride in oil	6/8	8/8	8/8	8/8				
Benzalkonium chloride	— [b]	0/8 (HS)	8/8	8/8				
Dibucaine hydrochloride vehicle					8/8	8/8	7/8	8/8
Dibucaine hydrochloride vehicle with no virus added					0/8	0/8	0/8	0/7
Propylene glycol					7/8	8/8	8/8	8/8
Soap					— [b]	0/6 (HS)	1/8 (HS)	8/8
Controls					31/32			

[a] Results are expressed as the number of mice reacting over the number inoculated. HS indicates that the difference from the controls is statistically highly significant.
[b] Deaths due to toxicity.

100 LD_{50} of CVS virus, incubated with agitation for one hour in an ice-bath and inoculated intracerebrally in test mice. All substances had previously been tested by the intracerebral route to estimate their toxicity and an effort was made to ensure that the greatest amount to be used was just tolerable to mice. Nevertheless, benzalkonium chloride and soap killed those test animals respectively that were given the 1:1000 and 1:5 dose. Little or no indication of virucidal effect was evident in those animals given dibucaine hydrochloride in saline, the dibucaine hydrochloride vehicle, dibucaine hydrochloride in oil or propylene glycol. However, both benzalkonium chloride and soap gave highly significant evidence of antiviral action. The mortality in the control animals was about 97%.

DISCUSSION

Although the value of prompt, adequate treatment by physicians of all wounds inflicted by rabid animals has been repeatedly stressed, little emphasis has been placed upon the role that first-aid procedures may play in disease prevention. The effectiveness of such simple and widely available substances as tap water, soap and water and soap solution in preventing rabies in animals with non-puncture wounds suggests that such treatment may be important in man when preferable therapeutic agents are not available or when delay is encountered in obtaining a physician. Since a large proportion of rabies exposures consist of mere scratches, minor lacerations or superficial bites, this type of treatment, subsequently reviewed and supplemented by a physician where indicated, might considerably reduce the risk of contracting rabies.

Thorough cleansing of the wound is most important irrespective of the therapeutic substance or substances to be used. Failure to reduce materially in these studies the hazard of rabies in animals whose deep puncture wounds were treated by swabbing with saline or superficial flushing with serum, for example, further emphasizes the need for adequate cleansing or debridement of infected wounds and the obvious advantage of using drugs with specific virucidal or other action such as benzalkonium chloride, soap solution and antiserum. It is noteworthy, however, that the wounds of the infected animals treated topically in this study received less adequate treatment than should be given to exposed humans.

The mode of action of rabies antiserum in preventing disease is an oft-argued matter. While the evidence seems clear that serum, when administered systemically in large doses, affords protection (Habel, 1945; Koprowski, van der Scheer & Black, 1950; Ishii, 1952; Ando et al., 1954), the effectiveness and method of action of locally administered antiserum are less obvious. Intramuscular infiltration around the area of the wound has been recommended (WHO Expert Committee on Rabies, 1960). Our studies indicate that marked protection may

also be achieved by thoroughly treating infected wounds with antiserum by deep flushing, flushing and swabbing, or a combination of intramuscular infiltration and swabbing, and that there is considerable protection afforded by swabbing with antiserum alone. Serum was much more effective when instilled in the wound than when inoculated parenterally; the protection was greatest when serum was administered within six hours of infection. Benzalkonium chloride, first used in rabies-infected wounds by Shaughnessy & Zichis (1943), equalled or outperformed antiserum in most experiments but often produced severe local tissue reactions when used in concentrations of 1%-2% to treat deep puncture wounds in guinea-pigs. Such reactions appeared largely attributable to retention of the drug within the wound. The excellent protection obtained by intensive local treatment with rabies antiserum one hour and even up to six hours after infection is in marked contrast with the results of Soloviev, Selimov & Kobrinski (1958), Kobrinski (1960) and Soloviev & Kobrinski (1962), who used powdered gamma-globulin and got little protection at one hour after infection and none thereafter.

In most cases of definite exposure in man, local treatment should be supplemented by the use of vaccine with or without administration of antirabies serum systemically, locally or both. Whenever antiserum is used, serious consideration should be given to its use locally as an adjunct to local treatment by intramuscular infiltration around the wound, topical application following adequate cleansing and debridement, or both.

The local anaesthetics used in these studies had little or no specific virucidal effect when inoculated systemically, although certain drugs exerted a marked sparing effect when inoculated intramuscularly proximally to the site of infection. The protection appeared greatest with the oil-base preparations and least or lacking with aqueous or saline-based local anaesthetics. For example, it is indicated that dibucaine hydrochloride in oil protected whereas dibucaine hydrochloride in saline did not. The evident ability of the dibucaine hydrochloride vehicle to induce a blocking effect suggests that such action is not attributable to specific virucidal action. Benzalkonium chloride is unique among the agents tested in that it apparently possesses both virucidal and blocking characteristics. It has been observed (Habel, 1941; Kligler & Bernkopf, 1943; Schindler, 1961) that virus inoculated intramuscularly in experimental animals rapidly diminishes in titre but may remain viable at the site of inoculation for 48-96 hours. Accordingly, as previously conjectured (Kaplan et al., 1962), blocking agents may impede the transmission of virus up the nerve trunk for a sufficient period of time to allow the virus titre to drop below the infectious threshold. This would explain the failure of saline to protect, the marked inhibitory effect of dibucaine hydrochloride in oil, and the intermediate action of propylene glycol. The mode of action of dibucaine hydrochloride in oil when used topically is not evident but may be due to a local blocking effect. How prominent a role, if any, blocking agents may play in preventing rabies in man is not presently known.

RÉSUMÉ

Bien que l'on ait depuis longtemps insisté sur la nécessité d'un traitement précoce de toutes les blessures provoquées par des animaux enragés, il ne semble pas que l'importance capitale des premiers soins ait été suffisamment reconnue. Ces premiers soins, le plus souvent très simples (lavage des plaies à l'eau du robinet, nettoyage à l'eau et au savon, application éventuelle de substances médicamenteuses), sont d'autant plus importants que d'une part les blessures ne sont souvent que de simples écorchures ou des morsures très superficielles et que d'autre part l'on ne dispose pas de véritable traitement spécifique de l'infection rabique déclarée.

Pour donner des preuves expérimentales de la valeur de ces soins locaux, les auteurs ont inoculé le virus rabique à un certain nombre d'animaux de laboratoire (cobayes et rats). Ces animaux ont été traités à différents intervalles de temps après l'inoculation et selon différentes méthodes, un groupe ne recevant aucun traitement. L'analyse statistique des résultats montre l'importance des soins locaux. C'est ainsi, par exemple, que du sérum antirabique est plus efficace lorsque appliqué directement dans les plaies que lorsque administré par voie générale.

L'on a particulièrement étudié l'effet favorable des substances susceptibles d'arrêter la migration du virus depuis le point d'inoculation. Il semble bien que les anesthésiques locaux oléosolubles de base et le chlorure de benzalkonium (Zephiran) qui ont un effet paralysant sur l'influx moteur favorisent grandement la résistance à l'infection. En ce qui concerne plus particulièrement le chlorure de benzalkonium, les expériences des auteurs montrent qu'il exerce une double action: il bloque la transmission nerveuse et détruit sur place le virus de façon spécifique.

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