THE PENETRATION OF AN ANTICHOLINESTERASE AGENT (SARIN) INTO SKIN. III. A METHOD FOR STUDYING THE RATE OF PENETRATION INTO THE SKIN OF THE LIVING RABBIT*

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In previous papers we have discussed the measurement of the rate of *in vitro* penetration of sarin into excised human abdominal skin (1) and into excised rabbit skin (2). The extreme toxicity of sarin makes *in vivo* experiments hazardous in man. In order to compare *in vitro* and *in vivo* penetration rates, we have developed a method for studying *in vivo* penetration of sarin into the skin of the living rabbit. This method is described in the present paper.

We have stressed the fact that in studies of skin penetration it is wise to determine by direct chemical measurement the amount of substance which has penetrated, and not to rely solely on observation of its biologic effect (1). The latter may give a false impression of the rate of penetration if only a small amount of substance produces a large biologic effect, e.g., mustard gas. In the method described here we can nevertheless make accurate use of a biologic effect of sarin, i.e., blood cholinesterase inactivation, because the per cent inactivation of blood cholinesterase is quantitatively related to the amount of active sarin which reaches the blood stream.

In rabbit blood, it has been shown that the inactivation of blood cholinesterase is directly proportional to the amount of an anticholinesterase agent added *in vitro* (3). We have found that sarin behaves similarly. One can safely administer sarin to a rabbit by intravenous infusion at the estimated rate of skin penetration. Sampling the blood at appropriate intervals permits determination of cholinesterase activity. From data so obtained, a calibration curve for each animal may be drawn showing the inverse relationship between blood cholinesterase and the amount of sarin administered. This calibration curve may vary for different rates of intravenous administration. The blood cholinesterase will completely regenerate in six to eight weeks, after which period *in vivo* penetration can be studied by applying a small amount of sarin to a known area of the rabbit's skin and determining the cholinesterase activity of blood samples taken at specified intervals. The amount of sarin which reaches the blood can be read directly from the calibration curve for cholinesterase activity versus the amount of sarin administered intravenously. From such data the rate of percutaneous penetration (mμM/cm^2·hr) can be calculated.

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This work was supported by the Army Chemical Corps, Contract No. DA 18-108-CML-4785.
Received for publication March 15, 1958.

METHOD AND RESULTS

The study of one animal is described in detail.

Sarin was added to a balanced electrolyte-dextrose solution (5 per cent dextrose with electrolyte No. 75; Parenteral Products, Mead Johnson & Co.) to make a concentration of 7.15 mμM/ml (1.0 μg/ml). At this concentration, very little sarin hydrolyzes while the solution is being injected. The solution was administered to a 3.7 Kg. rabbit at the rate of 13 drops (1.0 ml) per minute, through a polyethylene catheter inserted into the ear vein, the number of drops being recorded each minute. Blood samples (0.5–1.0 ml) were taken by cardiac puncture before the intravenous drip was started, and at approximately 4, 9, 20, and 30 minutes thereafter. The rate of administration and the exact time of sampling are shown in Table I.

The blood samples were analyzed for cholinesterase by the method developed by Fleisher *et al.* (3). The only alteration of their procedure was the substitution of physiological saline for 0.01 per cent saponin, as the saponin was shown to be unnecessary. The inactivation of cholinesterase by the various amounts of intravenously administered sarin is shown in Figure 1.

An interval of four months was allowed to elapse between the end of venous administration of sarin and the beginning of cutaneous administration. The rabbit weighed 3.9 Kg at the end of this period. Twenty-four hours prior to the beginning of percutaneous absorption, the abdomen was clipped; immediately before sarin was placed on the skin, the electrical conductivity of the experimental site was determined and found to be

TABLE I

The rate of intravenous administration
(13 drops = 1.0 ml = 7.15 mμM sarin)

Minutes	Drops	Remarks
1	24	
2	38	
3	53	
4	67	Sampled after 67 drops (37 mμM)
5	79	
6	93	
7	107	
8	121	
9	133	Sampled after 133 drops (73 mμM)
10	145	
11	157	
12	171	
13	184	
14	198	
15	210	
16	221	
17	235	
18	249	
19	265	
20	279	Sampled after 281 drops (154 mμM)
21	292	
22	305	
23	318	
24	330	
25	342	
26	355	
27	367	
28	381	
29	396	Sampled after 400 drops (220 mμM)
30	400	

low. We feel that a low conductivity indicates that the epidermal barrier is intact.

During the experiment, the rabbit, with the abdomen upward, was restrained on an operating board. A teflon sheet, 0.002 inch thick and measuring 4 cm x 4 cm, with a hole 3 mm x 3 mm in the center, was cemented to the abdominal skin with Duco cement. Approximately 2 μl sarin were placed on the 9 mm² of skin exposed at the center of the teflon sheet by means of a micropipette. A second unpunctured sheet of teflon was then immediately cemented on top of the first sheet in order to prevent evaporation of the sarin.

A sample of blood was taken by cardiac puncture just before the start of the experiment. Other samples were taken at 10, 20, 30, 45, 60, and 75 minutes after application of the sarin. The cholinesterase level was determined in the same way as after intravenous administration of sarin.

Table II and Figure 2 show the drop in blood

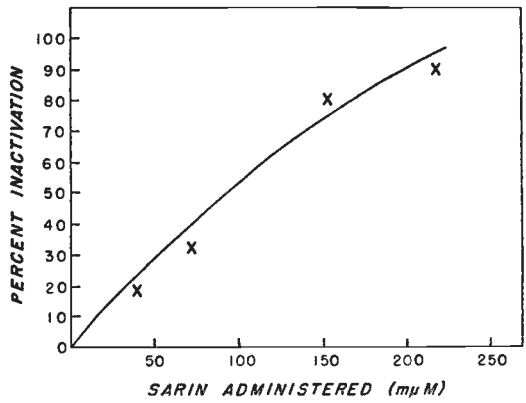

FIG. 1. Inactivation of cholinesterase by intravenous administration of sarin. Calibration curve.

TABLE II

The rate of inactivation of cholinesterase following percutaneous absorption of sarin

Time	Inactivation	Amount of Sarin Reaching the Blood Stream*
minutes	*per cent*	*mμM*
10	0	0
20	10	21.4
30	17	35.6
45	29	57.0
60	63	121.0
75	86	185.5

* Calculated from Figure 1 (Calibration curve)

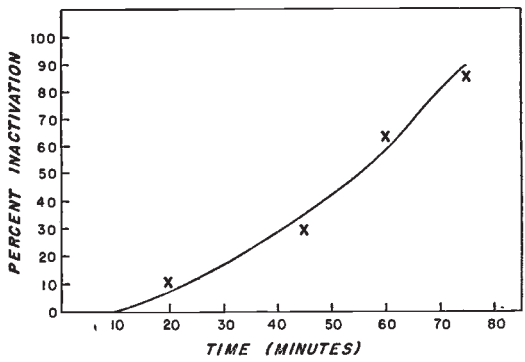

FIG. 2. Inactivation of cholinesterase by percutaneous absorption of sarin.

cholinesterase at various times following the application of sarin to the skin. The mμM of sarin necessary to cause the degree of inactivation observed can be determined from the calibration curve (Fig. 1). If one assumes that it required 15 minutes for the first sarin to penetrate the top layer of skin and reach the capillaries, and that

the rate of penetration was constant for the hour between 15 and 75 minutes, then 186 mμM (26 μg) of active sarin reached the blood stream through a 9 mm² area in one hour. The rate of penetration is therefore 2070 mμM/cm²·hr (290 μg/cm²·hr).

We have performed eight experiments of this type. The rate of *in vivo* penetration as determined in these eight experiments varies over a wide range, from about 357 to 3570 mμM/cm²·hr. This spread is due partly to biological variation and partly to technical errors.

Suggested Refinements of Technic

1. It is apparent from our data that the rate at which active sarin entered the blood stream was not the same in the intravenous as in the percutaneous observations. To avoid possible error, it seems wise to take the precaution of adjusting the rate of intravenous administration so that it corresponds very closely to the rate at which active sarin reaches the blood stream by the percutaneous route. If the sarin is administered too rapidly, an unknown proportion may escape into tissues other than the blood before it has exerted any effect upon the blood cholinesterase. If the two rates are as nearly identical as possible, this potential error will be minimized and will not seriously interfere with comparison of the two types of data. It should be possible to achieve this because the same animal can be used for several experiments if, at the end of a percutaneous experiment, the sarin remaining on the epidermal surface is immediately destroyed with alkali, or if the contaminated skin is excised.

2. Certain inaccuracies in the technic employed can be minimized by working with large rather than small animals. The larger the blood volume, the larger the total amount of sarin required to inactivate the cholinesterase, even though the per cent of inactivation per mμM of sarin may vary when two species of animals are used. Since the large animals can tolerate a larger amount of sarin, larger areas can be used for skin penetration and the percentage error caused by slight variations in the area of the skin can thus be reduced. The degree of trauma involved in taking blood samples would also be much less in the larger animal. Simple venipuncture is quite practical in the larger animals and is far less traumatic than cardiopuncture. In both large and small animals, trauma may be minimized and the blood sampling simplified by sampling through catheters inserted into the femoral vein.

3. Part of the sarin which penetrates the epidermis cannot be detected because a certain portion is inactivated by hydrolysis during penetration through the skin, and another portion remains in the lymph channels. Because neither of these portions can inactivate blood cholinesterase, the rates of penetration shown by experimental data in this paper are probably low. The use of radio-tagged sarin may help in the estimation of the amount of hydrolyzed sarin.

4. Variations in blood volume may occur during an experiment because of vasodilation due to the rise in concentration of acetyl choline as blood cholinesterase activity declines. In addition, variations in blood volume may result from growth during the interval between calibration of the animal and the beginning of the percutaneous absorption experiment. Sampling at the same time intervals in each experiment, choosing mature rabbits, and/or correcting for the increasing weight and blood volume may control this factor.

DISCUSSION

Despite its limitations and technical difficulties, this method for studying *in vivo* penetration allows preliminary comparison of *in vitro* and *in vivo* rates. The rate of *in vitro* penetration of sarin into excised rabbit skin ranged from 7 to 136 mμM/cm²·hr (2). (This was measured by an early technic which has subsequently been improved. However, the later more accurate *in vitro* technic (1) is applicable only to nonhairy skin.) The *in vitro* data cover a 4-hour period, the *in vivo* a 30-minute period. The values for the former were low because correction could not be made for the greater degree of hydrolysis of sarin during the 4-hour period. Further refinements in methodology, e.g., the use of larger animals, the use of femoral catheters to facilitate blood sampling, and the use of radio-tagged sarin for estimating the correction factor for hydrolysis of sarin are needed to increase the accuracy of the *in vivo* method reported here. No definitive comparison of *in vivo* and *in vitro* penetration should be attempted until both methods are refined.

SUMMARY

Methods which attempt to measure the cutaneous penetration of a substance by determining

its biological effect are seldom quantitative. For an anticholinesterase agent, however, the observed biologic effect (inactivation of blood cholinesterase) is a function of the amount of agent reaching the blood stream. This paper outlines a method by which the amount of an anticholinesterase agent reaching the blood stream through a unit area of skin in a unit time can be calculated after penetration has been allowed to occur into an animal previously calibrated by intravenous infusion.

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