TOXICOKINETICS OF HYDRAZINE ADMINISTERED PERCUTANEOUSLY TO THE RABBIT.

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the “Guide for the Care and Use of Laboratory Animals,” Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

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The percutaneous absorption of various aqueous concentrations of hydrazine over selected time periods has been studied. Duration of hydrazine exposure and hydrazine concentration correlated closely with the percent dose absorbed and peak serum hydrazine concentration. The data suggested that about 70% of the available hydrazine in H70 is still unabsorbed two minutes following hydrazine application. There was an increase in serum hydrazine concentration following removal of hydrazine from the skin surface suggesting the existence of an epidermal compartment.
Preface

This research was performed in the Toxicology Branch, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory from October 1980 through December 1982. It was performed in support of Project 6302, "Occupational and Environmental Toxic Hazards in Air Force Operations"; Task 630208, "Toxicology of Aerospace Fuels"; Work Unit 63020804, "Chronic Toxicology of Hydrazine Strategic Missile Fuels".

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INTRODUCTION

Hydrazine, \( \text{H}_2\text{NNH}_2 \), a polar, hydrophilic base, is a strong reducing agent with wide application in a variety of industrial processes. It is estimated that 90,000 workers are potentially exposed to hydrazine (Hz) or its salts each year (NIOSH, 1978). Hydrazine has been used in military and aerospace related systems as a propellant for many years. It was originally used as a jet fuel and a torpedo propellant during World War II in Germany, and more recently has been used in Titan II missiles, the emergency power unit (EPU) of the F-16, and the auxiliary power units of the space shuttle. The possibility that AF aircraft maintenance personnel may be subject to accidental Hz skin exposure is substantial. In addition, the proposed use of catalyzed aqueous solutions of Hz as corrosion inhibitors in naval propulsion boilers raises the possibility that Navy personnel may also be exposed to Hz.

Some data are available regarding skin exposure to Hz. The percutaneous absorption of Hz was described by Smith and Clark (1972) who applied Hz to the skin of anesthetized dogs at doses of 3-15 mmole/kg. A chemical burn occurred at the site of application, and absorption appeared complete by 70 minutes in all dose groups and sooner in the lower dose groups. The high dose group (15 mmole/kg) had a peak serum concentration of 60 \( \mu \text{g/ml} \) while the low dose group had a maximum serum concentration of 2 \( \mu \text{g/ml} \). The dermal LD\(_{50}\) for the dog was established as 90 mg/kg; for the rabbit, the dermal LD\(_{50}\) is 91 mg/kg, as compared to an iv LD\(_{50}\) of 20 mg/kg (Rotherberg and Cope, 1955). More recent research has been done concerning the percutaneous absorption of Hz in the rabbit (Keller et al., 1981) and this report relates the results of the initial phase of our study. It describes the skin absorption of Hz from a 70% Hz aqueous solution (H-70, used in the F-16 EPU) and from anhydrous Hz. Both anhydrous Hz and Hz from H-70 were found to be well absorbed percutaneously (86% and 55%, respectively).

This subsequent report describes the percutaneous absorption of Hz in the rabbit when exposures are terminated at various times (time-limited), and from more dilute aqueous solutions, 15% and 2%, than had been used before. The purpose of this research was to obtain additional data for the purpose of predicting the rate and extent of Hz absorption from anhydrous Hz, H-70, or more dilute aqueous Hz solutions. These additional data may also be used to test the reliability of predictions regarding percutaneous Hz absorption made in the initial report. Those predictions of bioavailability and absorption rate were made by assuming evaporation and percutaneous absorption of Hz from anhydrous Hz and H-70 applied to skin to be competing, first order processes (Keller et al., 1981).
METHODS AND MATERIALS

This study was accomplished in three parts: part one, reported previously (Keller et al., 1981), in which one group of rabbits received Hz intravenously, and two groups were percutaneously treated with Hz either as anhydrous Hz or H-70; part two, in which three groups of rabbits were percutaneously exposed to anhydrous Hz and three groups to H-70 and exposures terminated at two, six, or twelve minutes post application (time-limited exposures); and, part three, in which two groups of rabbits were percutaneously exposed to aqueous solutions of either 15% or 2% Hz.

Anhydrous Hz (95%, Eastman, Rochester, NY) was used for neat Hz administration and to prepare all aqueous Hz solutions except the 15% solution. This solution was obtained as Amerzine, a catalyzed 15% aqueous solution of Hz (Drew Chemical Corporation, Boonton, NJ). The Amerzine was tested to verify percent Hz in solution using a spectrophotometric technique described by Reynolds and Thomas (1964). All solutions except Amerzine were prepared just prior to administration to the rabbits.

All groups of rabbits, consisting of four or five New Zealand White rabbits (Willoughby's Rabbitry, Sabina, Ohio) weighing 2.9 to 3.9 kg, received 12 mg Hz/kg. The method of animal preparation and administration of Hz or Hz solutions was identical to that of part one (Keller et al., 1981). Briefly, this method consisted of placing a catheter in the femoral vein, clipping and then shaving an area of skin, applying the Hz or Hz solution to the shaved area, and taking serial blood samples which were analyzed for serum Hz concentration as described by Reynolds and Thomas (1964).

In part two, the percutaneous exposure to anhydrous Hz or H-70 was terminated at the appropriate time by flushing the treated area with dilute hypochlorite solution followed by a water rinse.

The group mean serum Hz concentrations were determined for each time period and serum Hz versus time was graphed. In addition, the area under the serum concentration time curve (AUC) was determined for each treatment group. The percent Hz dose absorbed for each treatment group was then determined by the equation, % dose = AUC percutaneous/AUC iv. The AUC was determined gravimetrically. The AUC iv and other data from the intravenous treatment group had been determined previously (Keller et al., 1981).

The rabbits were sacrificed 24 hours postexposure and selected hydrazine exposed skin sections were collected and examined histopathologically to determine any significant differences in skin damage with respect to Hz solution strength and/or time of exposure.

RESULTS

Time-limitation of percutaneous Hz exposure demonstrated a direct correlation between duration of exposure and peak serum Hz concentrations for both anhydrous Hz and H-70 Hz (figures 1 and 2). A correlation of
time to peak serum Hz level with duration of Hz exposure was also evident with anhydrous Hz but not H-70 Hz. Serum Hz levels peaked at 50-90 minutes for anhydrous Hz (Figure 1) and at 30-50 minutes for H-70 Hz (Figure 2). Total percutaneous Hz absorption from anhydrous Hz or H-70 as determined by percent
dose absorbed was also directly related to duration of Hz exposure (Figure 3). In addition, the rate of Hz absorption was greater for anhydrous Hz than for H-70 (Figure 3). The peak serum Hz concentrations for percutaneous exposure to 15% Hz and 2% Hz were 5.6 ug/ml and 3.1 ug/ml, respectively (Figure 4). These peak serum Hz concentrations occurred at 30 minutes for 15%
Severe chemical burns were found in rabbits percutaneously exposed to anhydrous Hz or H-70. These lesions typically contained areas of transepidermal necrosis with varying degrees of dermal necrosis. No significant lesions were noted following percutaneous exposure to 15% or 2% Hz solution.

**DISCUSSION**

A direct relationship was found between duration of percutaneous Hz contact and both peak blood concentrations of Hz and percent Hz dose absorbed through the skin for both anhydrous Hz and H-70 Hz (Figures 1, 2 and 3). These results clearly indicate that percutaneous Hz absorption, whether from anhydrous Hz or H-70 Hz, can be reduced by limiting the duration of exposure. Although the 12 minute absorption time yielded similar peak concentrations for H-70 and anhydrous Hz, the 6 minute absorption interval had a higher Hz serum concentration for H-70 and the 2 minute absorption interval had a higher Hz serum concentration for anhydrous Hz. It is apparent that the serum Hz-time curves derived from percutaneous absorption of anhydrous Hz are much broader, with a slower rate of decline, than the H-70 serum Hz-time curves. Thus, the AUC for serum Hz-time curve from anhydrous Hz absorption are larger and the percent doses absorbed are correspondingly larger (Figure 3). Also apparent (figures 1 and 2) is the time lag between percutaneous exposure to Hz and entry of Hz into the blood. All serum concentration versus time curves clearly show that blood Hz concentrations continue to increase long after Hz exposure has been discontinued. This phenomenon is more apparent with anhydrous Hz than with H-70 Hz. Since external Hz has been removed, this occurrence can only be explained by the existence of an epidermal compartment to which Hz moves from the external skin surface and from which it leaves to move into the blood. Thus, this second experiment
does seem to support the prediction made in the initial report that percutaneous Hz absorption is best explained by including an epidermal compartment. In addition, it is also possible the function of this epidermal compartment may be more complex with anhydrous Hz than with H-70 Hz due to the more intense interaction of Hz and tissue occurring with this more concentrated and caustic Hz solution. A faster movement of Hz from skin surface into the epidermal compartment followed by a slower but more extensive absorption into the blood would explain the broader serum Hz - time curves from anhydrous Hz skin exposures. Further support for the existence of an epidermal Hz compartment can be found by inspecting figure 3. The percent dose absorbed at each time interval is greater for anhydrous Hz than for H-70 Hz. In addition, the percent dose absorbed at 6 minutes was 36% for H-70 Hz and 42% for anhydrous Hz. Extrapolation to five minutes gives estimates of 30% for H-70 Hz and 39% for anhydrous Hz. This seems to suggest that the simple model of competing first order systems of evaporation and percutaneous absorption used in the initial report was not adequate for predicting anhydrous Hz percutaneous absorption, but was more adequate for predicting the absorption of Hz from H-70 (30% actual vs 24% predicted). In addition, these data suggest that models based solely on the rate of appearance of materials in the blood, rather than on the actual percent dose absorbed during time-limited studies, may be misleading with respect to prediction of rates of percutaneous penetration due to failure to take into account the epidermal compartment. By determining only the blood concentration - time curve, only the rate-limiting step for percutaneous absorption will be evident. In those instances when epidermal penetration rate is greater than distribution from the site of penetration (an epidermal compartment situation), a time limited study would seem to be the best approach for discerning the actual rate-limiting step of percutaneous absorption and the existence of an epidermal compartment. It would seem that the rate constants for percutaneous absorption of Hz from H70 and anhydrous Hz determined in our initial report are in fact rate constants for movement of Hz from the epidermal compartment to blood rather than the rate constants for Hz penetration of the stratum corneum. It remains to be seen whether this Hz epidermal compartment is the result of reversible epidermal binding, creating a temporary reservoir of Hz, or simply a function of difference between rate of Hz stratum corneum penetration and Hz diffusion from the local skin area into the blood. The requirement for an accurate estimate of the availability of an epidermal compartment for a percutaneous agent goes far beyond the academic desire to provide a complete toxicokinetic description for an agent. Time between exposure and peak blood levels such as those described for hydrazine are in fact similar to those involved in snake envenomation. The potential for effective local treatment to minimize Hz absorption is quite evident. Examination of data in Table 1 will allow estimates of the epidermal Hz compartment to be made. By assuming the total bioavailable Hz to be 100% and subtracting from it the percentage of Hz dose absorbed at time t divided by total bioavailable Hz absorbed, an estimate of the extent of the Hz epidermal compartment can be made (for H-70 at two minutes: 15%/55% = 27% and 100 - 27= 73% of bioavailable dose is available to the epidermal compartment). Thus 2/3-3/4 of the bioavailable percutaneous dose is still available for local treatment at two minutes.
Table 1

Percentage of Absorbed (Available) Dose Entering Epidermal Compartment Following Percutaneous Application of 12 mg Hz/kg as H-70 or Anhydrous Hz

<table>
<thead>
<tr>
<th>Time Interval (Minutes)</th>
<th>% Of Total Absorbed</th>
<th>% Remaining</th>
<th>% Of Total Absorbed</th>
<th>% Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-70 (55% Total Absorption)</td>
<td>Hz (87% Total Absorption)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-2</td>
<td>27</td>
<td>73</td>
<td>32</td>
<td>68</td>
</tr>
<tr>
<td>2-6</td>
<td>38</td>
<td>35</td>
<td>16</td>
<td>52</td>
</tr>
<tr>
<td>6-12</td>
<td>11</td>
<td>24</td>
<td>19</td>
<td>33</td>
</tr>
<tr>
<td>12-120</td>
<td>24</td>
<td>0</td>
<td>33</td>
<td>0</td>
</tr>
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</table>

Further inspection of Table 1 shows an apparent comparative slowing of percutaneous absorption for anhydrous Hz at 2-6 minutes. A physiologic explanation for this slower appearance of Hz in the blood during percutaneous absorption of Hz due to application of anhydrous Hz versus H-70 Hz may be found by examining the vasculature of the skin. Skin contains 3 levels of blood supply: a deep plexus, a middle plexus, and a superficial plexus of arteries with corresponding venous return. In addition, the lymph capillaries arise in the superficial dermis and drain into a subcutaneous plexus (Dellmann and Brown, 1981). Damage to the superficial and middle portions of skin by the more caustic anhydrous Hz could delay movement of Hz into the blood because the Hz would have to diffuse through the tissue to deeper venules, and perhaps a greater portion would also be cleared by the lymphatic system, which has a much slower circulation than blood.

The absorption of Hz from more dilute solutions has not been previously reported (Figure 5). No apparent local effect on the skin was visible following application of these dilute solutions of Hz as was reported for more concentrated Hz (Keller et al., 1981). Thus the absorption cannot be attributed to epidermal damage. This is a significant finding since many percutaneous occupational exposures to Hz are to more dilute solutions of Hz or perhaps even to Hz vapor. These data clearly demonstrate, however, that epidermal damage is not necessary for considerable percutaneous absorption to occur.

CONCLUSIONS

Our ability to confidently make recommendations regarding the hazard of human Hz exposures based on this study is limited by several factors: 1. Although we have corroborating data of a toxic effect following percutaneous Hz exposure (embryolethality in the rat, Keller et al., 1982) in another
species, no quantitative toxicokinetic data regarding percutaneous Hz absorption are available in a species other than the rabbit; 2. The number of animals involved in this study (4-5/group) is limited; and 3. Serum Hz was quantitated using a spectrophotometric technique which is unable to distinguish between Hz and monoacetylhydrazine, a known metabolite, leading to a "mixed" kinetic description. Despite these limitations, some recommendations can be made particularly regarding research, which will allow a more confident estimate of the percutaneous Hz hazard. Because of the finding of significant percutaneous absorption of Hz from dilute solutions, further work is needed to define the hazard of percutaneous absorption of Hz in dilute solutions or vapors and define the need for protective measures in various Hz work environments to enhance worker protection and efficiency. Because of the delay in appearance of Hz in the blood following percutaneous exposure, further work to define the effectiveness of local treatment may prove highly beneficial for developing techniques to treat Hz exposures. An adjunct to both of the above may be biological sampling of Hz-exposed workers via urine collection and analysis (Hershey et al.) as modified by George and Llewellyn (unpublished). Following establishment of baseline data on Hz in urine of exposed workers, sampling of this type might prove quite valuable for determining worker's actual daily Hz exposure as well as for diagnostic and prognostic tests for individuals with extensive Hz exposure.


REFERENCES


