The Absorption of Monomethylhydrazine through Canine Skin* (33846)

EDWIN B. SMITH AND DALE A. CLARK
Pharmacology-Biochemistry Branch, Biosciences Division, US Air Force School of Aerospace Medicine, Brooks Air Force Base, Texas 78235

The use of monomethylhydrazine (MMH) as a propellant fuel for rocket motors of space vehicles is attended by the danger of accidental exposure of personnel to this fuel. One of the most likely ways such an exposure might occur is by skin contact resulting from spillage or leakage of the fuel. However, most of the studies of the metabolic effects of hydrazine fuels have been made after administration of the fuel by inhalation, injection, or oral ingestion (1).

The present studies were therefore undertaken to ascertain how readily MMH is absorbed through skin and to observe selected metabolic effects of MMH after percutaneous absorption. Therefore, the appearance and behavior of the animals were recorded and the levels of MMH and of methemoglobin in the blood of anesthetized dogs were measured at intervals after topical application of known quantities of MMH.

Methods. Experimental protocol. Male mongrel dogs (10–15 kg) were fasted 12 hr, then anesthetized with pentobarbital, 30 mg/kg iv. Additional pentobarbital was injected as required to maintain the animals in deep anesthesia throughout the experiment. The femoral artery was exposed, and a polyethylene catheter was inserted for withdrawal of blood at intervals. Blood samples of 6 ml each were collected prior to the application of MMH and at the end of each time interval. Each time blood was withdrawn it was replaced with an equal volume of physiological saline. A 300-cm^2 medial area of the chest was shaved, and the desired volume of liquid MMH (reagent grade,[1] bp 87–89°) applied topically to the shaved area. A glass rod was used to spread the dose evenly over the area. To protect both the dog and the experimenters from inhaling the vapors, the dog was housed in a fume hood with the blower fan operating throughout the experiment. Experiments were terminated after 6 hr by an overdose of anesthetic.

Methemoglobin was measured by the spectrophotometric method of Evelyn and Malloy (2).

Plasma MMH. Arterial blood was collected in heparinized conical centrifuge tubes and centrifuged immediately. The plasma was promptly separated from erythrocytes to prevent disappearance of MMH caused by the red blood cells (3). MMH was then measured in a Technicon AutoAnalyzer using the automated method previously described (4). This determination is based on the light absorbed at 480 mμ after the reaction of MMH with excess *p*-dimethylaminobenzaldehyde in acid solution.

Under these conditions both hydrazine and MMH react readily. Dimethyl hydrazines and the hydrazones of glucose or of pyruvate give little or no color. The OD of the faint color produced by the reaction of the reagents with a control plasma sample obtained from the dog prior to application of MMH is subtracted from the OD of the reaction with all subsequent plasma samples. Levels of hydrazine or MMH added to plasma decrease slowly over a period of 4 hr, but in whole blood or red blood cells, the levels decline to very low values in 3 hr and remain low. The method therefore appears to measure only free nonmetabolized hydrazine or MMH.

Statistical analysis. The level of MMH in

---

* The research reported in this paper was conducted by personnel of the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks AFB, Texas. Further reproduction is authorized to satisfy the needs of the U. S. Government. The animals involved in this study were maintained in accordance with the "Guide for Laboratory Animal Facilities and Care" as published by the National Academy of Sciences—National Research Council.

[1] Matheson, Coleman and Bell, Division of the Matheson Co., Inc., Norwood, Ohio.

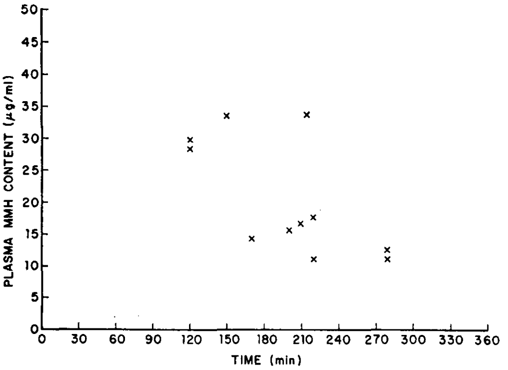

FIG. 1. Relationship between plasma MMH content and time to onset of convulsions in anesthetized dogs after cutaneous application of MMH.

plasma and the percentage of hemoglobin converted to methemoglobin were expressed as a function of dose of MMH using the method of least squares to calculate the best straight line relationship (5). Such a least squares fit was computed for each sampling time.

Results. Application of MMH caused marked discoloration of the skin wet by the compound. This area was noticeably reddened within 5 min. The color deepened and changed progressively from dark red to yellow-brown to dark brown-purple over a period of 3 hr. The color then faded until, by 6 hr after application of MMH, the site appeared slightly gray. Marked edema of the skin also developed over the area where MMH was applied. Swelling became very evident within 10–20 min. After 3 hr it began to decrease and disappeared within 3 more hr.

As with other routes of exposure, MMH applied to the skin caused convulsions, even in anesthetized dogs. Convulsions were typically initiated by mild spasmodic abdominal muscle twitchings which progressed to generalized clonic seizures during a period of 10–12 min. The onset of convulsions appeared to require a plasma MMH level of at least 10 μg/ml. As Fig. 1 shows, the time of onset of convulsions appeared to vary with plasma level of MMH. In general, the higher the level of MMH, the shorter the time to the onset of convulsions. However, one dog reached a level of 37.5 μg of MMH/ml, but was never observed to convulse. As Fig. 2 shows, this relationship appears more consistent when the size of dose is plotted against time to onset of convulsions. In any given animal, the severity of convulsions appeared to increase as the level of MMH in the plasma rose. However, when convulsions in different animals were compared, the severity did not correlate with either plasma MMH level or dose of MMH applied. Since all the experimental animals were kept well anesthetized, the significance of these observations of the relationship between plasma MMH levels and the occurrence of convulsions is uncertain.

The MMH applied to canine skin was rapidly absorbed into the blood stream and was detected in the plasma of blood samples withdrawn from the femoral artery within as little as 30 sec after application of MMH on the shaved chest. The level of MMH in the blood then rose as shown in Fig. 3. After low doses of MMH (<3 mmoles/kg) the level peaked around 60 min after application and decreased slowly thereafter. As the dose increased, the time required to reach peak blood levels increased and the rate of subsequent decline decreased until, with doses of 4–6 mmoles/kg, no peak was reached in the 6-hr observation period.

The plasma MMH level at each of 11 sampling times was analyzed as a logarithmic function of dose according to the equation $\log y = a + bx$, where $\log y$ = log plasma MMH, x = dose MMH, a = intercept, and b = slope of dose–response curve.

The blood MMH values for each sample time for 15 MMH-treated dogs were an-

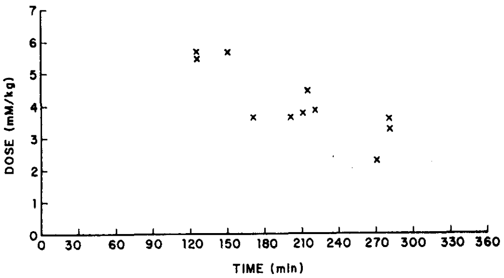

FIG. 2. Relationship between time to onset of convulsions and size of dose of MMH applied to skin of anesthetized dogs.

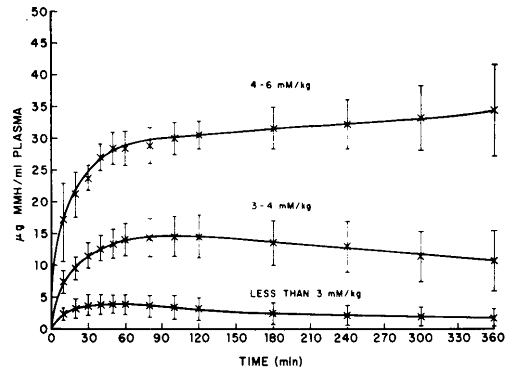

FIG. 3. Plasma monomethylhydrazine level in anesthetized dogs after cutaneous application of various doses of MMH: each point is the mean of the indicated *n* values for the dosage groups at the indicated time; bars enclose ± one standard deviation, which includes both animal variability and the response variability resulting from the spread of dose levels. Actual doses administered (mmoles/kg) were: 0.32, 0.59, 1.11, 2.47, 3.30, 3.32, 3.59, 3.67, 3.67, 3.78, 3.91, 4.47, 4.98, 5.54, 5.73, and 5.75.

alyzed in this way and the results are summarized in Table I. Since the data in Fig. 3 show that the blood MMH levels vary with time after application of MMH, the increase in values for the slope of the dose–response curve with time is reasonable. The pattern of change of the intercept *a* with increasing time appears provocative, but its significance is unknown.

The percentage of blood hemoglobin converted to methemoglobin at intervals after application of various doses of MMH to the skin of dogs is shown in Fig. 4. Obviously the methemoglobin content of blood increased during approximately 2 hr and decreased gradually thereafter. At any given time the level appeared to vary directly with the dose of MMH applied.

TABLE I. Regression[a] of Log Plasma MMH Level (μg/ml) on Dose at Various Times after Application of MMH to Canine Skin.

Time (min)	Intercept (*a*)	Slope (*b*)
10	0.168	0.192
20	0.287	0.191
30	0.357	0.191
40	0.373	0.198
50	0.385	0.200
60	0.367	0.207
120	0.238	0.241
180	0.083	0.273
240	0.022	0.285
300	—0.080	0.306
360	—0.144	0.319

[a] Log $y = a + bx$; where log $y =$ log plasma MMH, $x =$ dose MMH.

The dose–response relationship between MMH applied and methemoglobin levels subsequently observed was anlayzed in the same way as was done for blood MMH levels. The results are summarized in Table II. The variation of slope of the dose–response curve with time appears reasonable in view of the relationships demonstrated in Fig. 4.

TABLE II. Regression of Log Blood Methemoglobin Concentration on Dose of MMH and Correlation of Methemoglobin Levels with MMH Levels at Various Times after Application of MMH to Canine Skin.

	Regression lines[a]		
Time (min)	Intercept (*a*)	Slope (*b*)	Correlation coefficients
10	—0.812	0.224	0.98
20	—0.291	0.172	0.97
30	—0.199	0.189	0.97
40	—0.155	0.200	0.95
50	—0.394	0.274	0.96
60	—0.409	0.284	0.96
120	0.162	0.180	0.96
180	0.165	0.173	0.94
240	0.036	0.192	0.95
300	—0.036	0.195	0.94
360	—0.255	0.228	0.92

[a] Log $y = a + bx$, where log $y =$ log blood methemoglobin expressed as % of total hemoglobin, and $x =$ dose MMH.

A relationship of blood methemoglobin levels to MMH levels is suggested by the general similarity in the shapes of the curves of Figs. 3 and 4. However, methemoglobin levels differ from MMH levels in two significant ways: (i) peak levels of methemoglobin occur within 100–140 min regardless of dose of MMH, and (ii) these levels decline thereafter, even after large doses of MMH which produce a continually rising level of blood MMH. Despite these differences, however, the correlation between plasma MMH

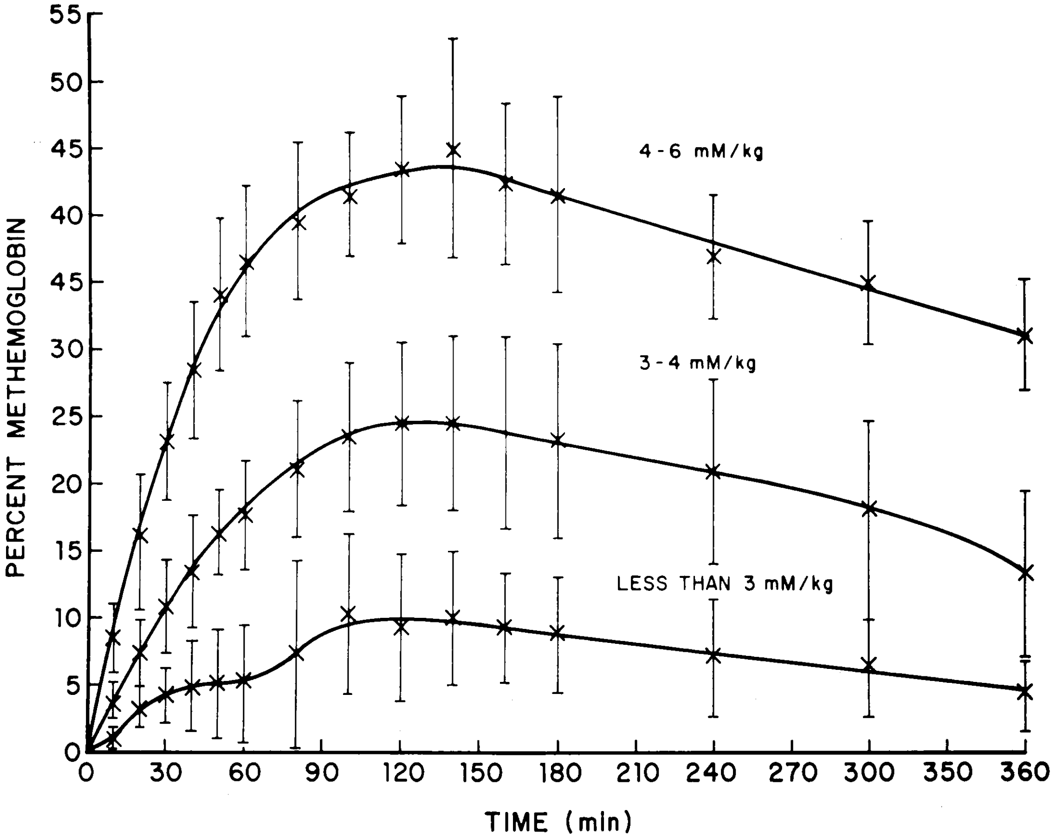

FIG. 4. Plasma methemoglobin level in anesthetized dogs after cutaneous application of various doses of MMH: additional details are the same as given in the legend of Fig. 3 except that zero methemoglobin levels after the 0.32-mmoles/kg dose were not included in the data analysis.

and methemoglobin levels was high ($r \geq 0.92$) as evident in the right-hand column of Table II.

Discussion. Of the methyl hydrazines, the most toxic is MMH (6), for which the LD$_{50}$ by iv injection was reported to be 0.26 mmoles/kg for male mongrel dogs observed over a 10-day period (7). In our studies of percutaneous absorption of MMH, of 16 dogs that received doses ranging from 0.3 to 5.8 mmoles/kg, only one dog died. It had received a dose of 3.8 mmoles of MMH/kg. However, these animals were anesthetized and, therefore, protected to at least some degree from the toxicity of MMH (8). In addition, the observation period after application of MMH was only 6 hr. Since severe intoxication was evident at that time in most of the animals, the mortality during a 10-day observation period such as Witkin's undoubtedly would be much greater (7). The LD$_{50}$ of MMH applied to canine skin therefore cannot be estimated from our observations.

Percutaneous absorption of MMH was studied by Rothberg and Cope (9), who found that the LD$_{50}$ dose for 24-hr survival was 1.05 and 2.07, respectively, for guinea pigs and rabbits. The LD$_{50}$ dose for guinea pigs by iv injection was not established, but for rabbits it was 0.27 mmoles/kg. The percutaneous route therefore increased the LD$_{50}$ dose approximately 7-fold in that species. Even with this increase the toxicity of MMH absorbed through the skin is still great. This fact is indicated in our studies by the severe intoxication seen in anesthetized animals during only 6 hr of observation.

Despite anesthetization, convulsions occurred in 11 of the 12 dogs receiving doses greater than 3 mmoles of MMH/kg. The conclusion appears warranted that MMH is quite readily absorbed through skin, or at least through canine skin. This fact is underscored by the observation that MMH applied to the dog's shaved chest was detectable in blood drawn 30 sec later from the femoral artery.

In their studies with rabbits, Rothberg and Cope (9) observed that MMH applied to the skin caused mild edema in the treated area. After 24 hr the edema was gone, but the affected area of skin had a blanched appearance. In our dogs, the area where MMH was applied appeared to suffer a severe chemical burn. The edema was quite pronounced, and the sequence of skin discolorations was similar to those seen after a severe bruise. These discolorations suggest that MMH caused stasis of blood and breakdown of red cells and of hemoglobin in the treated area.

Previous investigations have established that iv injections of MMH produce methemoglobin levels that vary with species but are highest in dogs (10). It is therefore not surprising that percutaneous absorption of MMH also produces methemoglobinemia in dogs. Comparison of the methemoglobin levels in dogs after iv and percutaneous administration of MMH provided another estimate of the relative effectiveness of a given dose of MMH administered by these routes. Since a dose of only 0.54 mmoles/kg injected iv produced a peak methemoglobin level greater than 30% (3), it appears that the dose applied to the skin would have to be 5–7 times larger than the dose injected iv to produce comparable peak levels of blood methemoglobin. This factor agrees well with the larger dose required to cause 50% mortality after cutaneous application than after iv injection.

The peak methemoglobin levels occurred about 1 hr after iv injection of MMH in the dog, but peak levels after application to the skin occurred in approximately 2 hr. By the latter route, however, maximum blood MMH levels were not reached until approximately 1 hr had elapsed; whereas after iv injection, maximum blood MMH levels occurred within 3 min. Apparently peak methemoglobin levels occurred approximately 1 hr after peak MMH levels were reached, whether the MMH was injected or applied to the skin. An obvious exception to this generalization, however, is the continually rising blood MMH level found in dogs after application of large doses of MMH to the skin.

The occurrence of methemoglobinemia may be related to effects of MMH on catalase activity. Blaschko (11) noted that substances which cause reversible formation of methemoglobin also inhibit catalase. Studies of Cohen and Hochstein (12) indicated that peroxide accumulation results in methemoglobin formation. Since hydrogen peroxide is produced by autoxidation of certain MMH derivatives (13) and hydrazine has been reported to inhibit catalase (11), it seems likely that MMH may inhibit catalase in the erythrocytes and/or cause peroxide levels to rise. The latter, in turn, should increase production of methemoglobin and cause methemoglobinemia. If this is the mechanism, it would follow that as blood MMH levels rose (or fell), methemoglobin levels should also rise (or fall). In general, such a relationship was observed in dogs treated with less than 4 mmoles of MMH/kg.

However, there was a lag between the appearance of MMH and of methemoglobin in the blood. Methemoglobin was not elevated in blood in less than 3 min after application of MMH to the skin, although MMH was measurable within 30 sec. This lag could represent the time required for the blood MMH to rise to a sufficiently high level for methemoglobin to be formed. The other lag, the 1 hr lag of peak methemoglobin levels behind peak MMH levels, could represent the time required for the rate of methemoglobin formation to fall below the rate of methemoglobin reduction. These lag times, therefore, appear to be compatible with a mechanism of methemoglobin production based on inhibition of catalase by MMH.

A further point is also compatible with that mechanism, which would predict that as peak levels of blood MMH increased with higher doses of MMH, greater inhibition of catalase should occur. Therefore, peak levels of methemoglobin should also increase with increasing doses of MMH. This relationship was demonstrated by the data and supports the postulated mechanism.

One important observation, however, does not fit the postulated mechanism. In dogs treated with large doses of MMH, the blood levels of MMH continued to rise, while methemogloglobin levels reached a peak and decreased thereafter. If inhibition of catalase by MMH were the sole cause of methemoglobin accumulation in the blood, then continued elevation of MMH levels should have resulted in continued elevation of methemoglobin levels. The observed decrease in methemoglobin levels under those conditions means that the postulated mechanism is either incorrect or incomplete. Perhaps additional factors come into play and affect methemoglobin reductase. Further clarification of these mechanisms is needed.

Apparently chronic intake of low doses of hydrazine can be tolerated indefinitely, or until the rate of intake exceeds the body capacity to detoxify and/or excrete the hydrazine (1). If the same is true for MMH, the data presented above imply that within 2 hr after doses less than 4 mmoles/kg, the rate of absorption through the skin had decreased to the point where it was equal to the rate of detoxication and/or excretion. Thereafter, blood levels of MMH fell because presumably the rate of removal exceeded the rate of input. With sufficiently large doses of MMH, the rate of input apparently never fell below the rate of output. Doses sufficient to produce blood MMH levels higher than 25 μg/ml (25 mM) invariably resulted in a pattern of continuously rising levels during the 6-hr observation period. It is inferred that this level of MMH was high enough to embarrass metabolic systems of critical importance in detoxication and/or excretion so that removal of MMH could not equal absorption. The result was a continuous increase in blood level of MMH.

Failure of kidney function is a likely contributor to this critical change, for several observations have been made that would support such an inference. It was found that administration of MMH to dogs caused intravascular hemolysis (14), methemoglobinemia (3, 15), and bilirubinemia (6). As a species, dogs were found to develop the highest levels of methemoglobin (10) and to be peculiarly subject to renal change (16) after injections of MMH.

All these observations suggest a metabolic sequence involving methemoglobin formation resulting in hemolysis and increased formation of bile pigment. The increased load of heme pigments to be excreted could contribute to, if not actually cause, the renal damage produced by MMH. Failure of excretory function therefore seems to be a likely explanation for the continual rise in blood levels of MMH after cutaneous application of large doses. The implication of this continuing rise is that, even if protected from the effects of MMH on the central nervous system, few dogs would recover spontaneously from cutaneous doses of MMH larger than 4 mmoles/kg because the ensuing high blood levels of MMH apparently cause metabolic effects that cripple normal detoxication and/or excretion mechanisms.

Summary. Graded doses of monomethylhydrazine (MMH) were applied to a 300-cm^2 area of the shaved chest of anesthetized dogs. The area of skin to which the MMH was applied was reddened within 5 min, and the color progressively changed to deep red, then to yellow-brown to dark purple and, after 6 hr, to light gray. The area of application swelled markedly within 10–20 min. Swelling persisted for 3 hr, then gradually decreased and disappeared by 6 hr. MMH was detectable in the blood within 30 sec after the initial application. With doses less than 3 mmoles/kg, blood MMH levels rose to a peak in approximately 1 hr, and decreased slowly during the next 5 hr. As size of dose increased, higher peak levels of blood MMH were reached and the rate of subsequent decline decreased until, with doses of 4–6 mmoles/kg, no decline was seen during the 6-hr period of observation. Within 3–5 min after application of large doses of MMH to

the skin, methemoglobinemia was observed. Levels of methemoglobin rose to a peak at about 2 hr, then declined slowly. Peak levels varied directly with the dose of MMH applied, and blood levels of methemoglobin and of MMH were closely correlated throughout the observation period. Peak methemoglobin levels were approximately 1/5 of peak levels seen after iv injection of equal doses. The absence of a decline in blood MMH levels after large doses was attributed to effects of MMH on metabolic systems of detoxication and/or excretion.

---

1. Clark, D. A., Bairrington, J. D., Bitter, H. L., Coe, F. L., Medina, M. A., Merritt, J. H., and Scott, W. N., USAF School of Aerospace Med. Aeromed. Rev. 11–68, in press.
2. Evelyn, K. A. and Malloy, H. T., J. Biol. Chem. **126**, 655 (1938).
3. Fortney, S. R. and Clark, D. A., Aerospace Med. **38**, 239 (1967).
4. Smith, E. B. and Korty, P. R., USAF School of Aerospace Med. Tech. Rept. **68-36**, 1968.
5. Li, J. C. R., "Statistical Inference," Vol. 1, Chap. 16 and 17, pp. 279–354. Edwards Brothers, Ann Arbor, Michigan (1964).
6. Jacobson, K. H., Clem, J. H., Wheelwright, J. H., Rinehart, W. E., and Mayes, N., A.M.A. Arch. Ind. Health **12**, 609 (1955).
7. Witkin, L. B., A.M.A. Arch. Ind. Health **13**, 34 (1956).
8. Medical College of Virginia report to Army Chem. Corps, Med. Lab. Contract Rept. MLCR No. **62**, (1955).
9. Rothberg, S. and Cope, D. B., Chem. Warfare Lab. Rept. CWLR **2027**, (1956).
10. Clark, D. A. and DeLaGarza, M., Proc. Soc. Exptl. Biol. Med. **125**, 912 (1967).
11. Blaschko, H., Biochem. J. **29**, 2303 (1935).
12. Cohen, G. and Hochstein, P., Biochemistry **2**, 1420 (1963).
13. Berneis, K., Kofler, M., and Bollog, W., Experientia **20**, 73 (1964).
14. Medical College of Virginia report to Army Chem. Corps, Med. Lab. Contract Rept., MLCR No. **52**, (1955).
15. Van Stee, E. W., Aerospace Med. **36**, 764 (1965).
16. Pinkerton, M. K., Hagan, E. A., and Back, K. C., Toxicol. Appl. Pharmacol. **10**, 401 (1967).

Received Nov. 27, 1968. P.S.E.B.M., 1969, Vol. 131.