WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTÉ

WHO/SE/71.29

ENGLISH ONLY



Later published in "Am J Epidemiol" 94: 435-49, 1972

# FIELD TRIALS OF METHISAZONE AS A PROPHYLACTIC AGENT AGAINST SMALLPOX[a]

by

Gordon G. Heiner, Nusrat Fatima, Philip K. Russell, Ashley T. Hasse, Nur Ahmad, Niaz Mohammed, David B. Thomas, Thomas M. Mack, Malik Muzaffar Khan, Genell L. Knatterud, Ronald L. Anthony and Fred R. McCrumb, Jr.

Three field trials of methisazone as a prophylactic agent against smallpox were carried out in West Pakistan between 1964 and 1970. Subjects were household and compound contacts of smallpox cases, assigned to drug or placebo groups by previously randomized schedules. Attack rates were 2.7 per cent. (seven of 262 contacts) and 5.0 per cent. (13 of 260 contacts), respectively, in the drug and placebo groups, a difference that was not statistically significant. However, there was a suggestion of slightly greater drug effect among contacts unvaccinated prior to exposure. No differences in morbidity or mortality were noted between cases in the two groups. The third trial was a double-blind study, in which attack rates were 3.8 per cent. (four of 106 contacts) and 4.9 per cent. (five of 103 contacts) in the drug and placebo groups respectively. Comparison of paired sera of 13 contacts in the drug group and 12 in the placebo group provided no evidence of a suppressive effect of methisazone on the immunologic response to vaccination. Results of previous studies of methisazone have been compared.

The activity of thiosemicarbazones against vaccinia and variola viruses has been studied extensively during the past two decades.[1-8] One of these compounds, methisazone (1-methylisatin, 3-thiosemicarbazone) (Marboran) was found to have a marked protective effect in variola-infected mice when given during the incubation period,[8] and the drug was subsequently developed as a possible prophylactic agent in man.

Two major trials of the prophylactic efficacy of methisazone have been conducted. Bauer and associates,[9,10] carried out a field evaluation of the drug among household contacts of smallpox cases in Madras, India, in 1963 and found that it exerted a significant protective effect. Do Valle and his co-workers[11] conducted a trial among contacts of cases of variola minor in São Paulo, Brazil, in 1964-65, with similar results. A more limited study by Rao et al.[12] in Madras during the period 1965-67 failed to demonstrate a significant protective effect of methisazone among unvaccinated contacts, but the numbers involved were small.

Three consecutive field trials of methisazone were conducted by the Pakistan Medical Research Centre between 1964 and 1970, the cumulative results of which are herein reported.

---

[a] From the Pakistan Medical Research Center (University of Maryland International Center for Medical Research and Training), Lahore, West Pakistan and the Division of Virus Diseases, Institute of International Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201.

This study was supported by United States Public Health Service Grant AI10049 entitled "International Center for Medical Research and Training" and by Grant AI08544 under the auspices of the United States-Japan Co-operative Medical Science Program, administered by the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

METHODS

The basic plan of the three trials was to identify contacts of active smallpox cases, administer methisazone or placebo in a pre-determined random sequence and determine any protective effect of the drug in reducing the incidence of disease among the contacts. Study 1 (1964) and Study 2 (1965) were conducted as pilot projects. Study 3 (1966-70) differed principally in the introduction of double-blind procedures for the administration of the drug. Other variations in protocol are discussed below.

The trials were conducted in Punjab Province of West Pakistan, in the cities of Lahore, Gujranwala and Sialkot and in rural villages within a radius of 250 miles of Lahore. Outbreaks were located through notification by local District Health Officers or by follow-up of epidemiological leads from earlier outbreaks.

Index cases and contacts. An index case was defined as the first case of smallpox in a house or compound. Only those cases were accepted in which the onset of rash had occurred 12 days or less before the first visit of the investigating team. The first day of rash of the index case was considered to be the first day of exposure of contacts.

A contact eligible for the study was defined as any person sleeping regularly in the same house or compound as the index case during the period of his illness. In a Punjabi village, a typical house consists of one or two small rooms without windows. A compound is composed of two or more such houses with a common walled courtyard, which in most cases are occupied by relatives. City dwellings usually have a comparable degree of crowding within the household, but there normally is no equivalent of the village compound.

Index cases were identified on the first visit of the investigators, all household and compound contacts were registered in order of descending age and a questionnaire was completed for each contact. The questionnaire included demographic data on the contact, vaccination scar status, and a record of vaccination history, previous smallpox and duration of exposure to the case.

The following contacts were excluded either during the field study or in the subsequent analysis:

(1) Persons with a history of previous smallpox or of variolation, who were considered to be essentially not at risk;

(2) Persons to whom drug was administered after the onset of rash subsequently diagnosed as smallpox;

(3) Fifty-seven persons in Study 2 for whom basic data were incomplete. Of this group, 27 had received methisazone and 28 placebo, while two had no drug; there were no secondary cases among them.

Drug administration. Registered contacts were assigned to the methisazone or placebo group in accordance with a previously randomized schedule, and drug was administered accordingly. During the period of Study 3, double-blind procedures were used. For each dosage level, methisazone and placebo were bottled in identical individual containers, randomized and numbered sequentially by a person not engaged in the field operations. Bottles were then dispensed to contacts in numerical order, and the drug consumed in the presence of the investigators.

Adult contacts in Study 1 received two doses of methisazone, each consisting of 3 g of drug suspended in 15 ml of a sucrose vehicle, or two doses of placebo in comparable volume. The doses were given at intervals of four to six hours, and both were taken in the presence

of the investigators. Adult contacts in Studies 2 and 3 received a single dose of 6 g of drug. In all studies, children were given reduced amounts. In Studies 1 and 2, children of less than five years received one half of the adult dose. In Study 3, the following dosage schedule was used:

| Age (years) | Dose (grams) |
|---|---|
| < 1 | 1 |
| 1-4 | 2 |
| 5-9 | 3 |
| 10-14 | 4 |
| ≥ 15 | 6 |

Methisazone in a 20 per cent. syrup suspension was supplied by the Wellcome Laboratories of Tropical Medicine, Beckenham, Kent, England. A suspension of a similar yellow colour was used as placebo.

Vaccination. A majority of contacts in Studies 1 and 2 were vaccinated by local public health personnel during the outbreaks, and the time and outcome of such vaccination were recorded by the investigating team. During the period of Study 3, all contacts were vaccinated primarily or revaccinated by the investigators at the time of drug administration, unless already vaccinated by the local authorities. These differences in post-exposure vaccination are considered in the analysis of results. Throughout the period of these studies, liquid calf-lymph vaccine was employed by local public health authorities, while freeze-dried vaccine reconstituted prior to use was employed by the investigators.

Subsequent visits to contacts were scheduled to assess vaccination results and to identify secondary cases of smallpox among the contacts. A second visit was made 5-10 days after the first, and a final visit was made after at least 14 days in Studies 1 and 2 and after 40-70 days in Study 3.

Serologic studies. During the period of Study 3, baseline serum specimens were collected when contacts were admitted to the study, in order to determine residual antibody levels of those contacts who subsequently developed disease. Whenever possible, a second specimen was obtained 40-70 days after the first, in order to permit an evaluation of the effect of methisazone on the response to vaccination. Serum specimens were stored at -20°C until serologic tests were conducted.

Complement-fixation (CF) and haemagglutination-inhibition (HI) tests were performed by a micro-adaptation of the procedures outlined by Kempe & St. Vincent.[13] CF titres were reported as the reciprocal of the highest dilution of serum which fixed two exact units of complement in the presence of four units of soluble antigen derived from variola-infected cell culture.[14] HI titres were recorded as the reciprocal of the highest dilution of serum contained in 0.025 ml capable of inhibiting the agglutinating activity of 0.025 ml of variola haemagglutinin[15] containing two HA units.

Passive haemagglutination (PHA) tests were performed using human "O" erythrocytes sensitized with variola-specific soluble antigens.[16] PHA titres were recorded as the reciprocal of the highest dilution of serum capable of agglutinating 0.025 ml of sensitized erythrocytes.

Virus neutralization tests were performed with vaccinia virus, using a plaque reduction technique.[17] Titres of virus neutralizing (NT) antibody were recorded as the reciprocal of that dilution of serum effecting a 50 per cent. reduction in the number of plaques observed in a virus control.

International Standard Anti-Smallpox Serum was used as a positive control in each test.

Statistical analysis. The chi-square test was used generally in the determination of significance of differences in the composition of study subgroups and in attack rates in treatment groups. However, whenever one of the expected frequencies was less than 5, the Fisher exact probability test was employed. A p value of less than .05 was considered significant.

RESULTS

Ninety-seven, 220 and 253 contacts were entered into Studies 1 (1964), 2 (1965) and 3 (1966-70), respectively, representing an overall total of 570 persons. Distribution of contacts and of subsequent cases by treatment category is presented in Table 1. The methisazone-treated group numbered 262 and the placebo group 260. In addition, 26 persons refused treatment and no treatment was given to 22 others, of whom 13 were absent at the time of drug administration and nine were excluded by error.

Within the methisazone-treated group of 262 contacts, 10 consumed less than a full dose of drug, three had less than a full dose and later vomited and 20 had a full dose but subsequently vomited. Among these 33 contacts, many consumed and retained a major portion of the dose given. Only one secondary case occurred among them, a one-year-old boy who vomited eight hours after administration of the drug, presumably after absorption had occurred. Accordingly, all have been included in the treated group for further analysis. The placebo group of 260 similarly includes 15 contacts who consumed less than a full dose, one who had less than a full dose and vomited and three who consumed a full dose but later vomited.

Composition of the methisazone and placebo groups was comparable by the parameters of age, sex and pre-exposure vaccination status (Tables 2 and 3). Vaccination status was defined as presence or absence of vaccination scars. In each of the three separate studies, drug and placebo groups were similarly analysed and no significant differences were found.

A total of 21 cases of smallpox occurred among the contacts in the study. Two of these were presumed to be co-primary cases, since the periods between the onset of rash of the index case and onset of illness in the contacts were five or less and seven days, respectively. Two of the cases, including one of the co-primary cases, were febrile at the time of the first visit; one received methisazone and one refused treatment. These have been included in the analysis, since they were entered into the study during the prodromal period. However, it is clear that for the one case who received methisazone, administration of drug may have been too late to have exerted any effect.

All but two of the 21 cases occurred in children of 10 years of age or less, the two exceptions being a vaccinated 18-year-old boy and an unvaccinated 28-year-old woman. Fifteen of the cases were males and six were females. Fifteen cases occurred among 50 contacts unvaccinated prior to exposure, and six cases among 510 previously vaccinated persons.

Attack rates in treatment groups. Seven cases of smallpox occurred among 262 contacts in the methisazone group, representing an overall attack rate of 2.7 per cent., compared to 13 cases among the 260 contacts of the placebo group, for an attack rate of 5.0 per cent. (Table 1). The difference was not significant at the five per cent. level by the chi-square test. In the double-blind study, the difference was even smaller; attack rates in the drug and placebo groups were 3.8 per cent. (4 cases among 106 contacts) and 4.9 per cent. (5 cases among 103 contacts), respectively.

No differences in morbidity or mortality were noted between cases who had been given drug and those who received placebo. There was one death among the seven cases in the methisazone group, and two among the 13 cases in the placebo group. Of the survivors, there were five mild or moderate cases and one severe case in the drug group, and 11 mild or moderate and no severe cases in the placebo group.

Among previously vaccinated contacts, there was essentially no difference between the methisazone and placebo groups, the respective attack rates being 1.2 and 1.3 per cent. (Table 4). However, among contacts unvaccinated prior to exposure, attack rates in the drug and placebo groups were 22.2 per cent. (4 cases among 18 contacts) and 45.5 per cent. (10 cases among 22 contacts), respectively. This difference was not significant by the chi-square test, but the numbers were small.

Two basic dosage regimens were used in the trials. Attack rates by equivalent adult regimen are shown in Table 5. Contacts who received two doses of 3 g, given at an interval of four to six hours, or the equivalent reduced dose for children, showed an attack rate of 2.2 per cent., while those who received a single dose of 6 g or the equivalent reduced dose had a rate of 2.8 per cent. Neither differs significantly from the attack rate in the corresponding placebo group.

Attack rates by duration of exposure prior to administration of drug are presented in Table 6. The usual incubation period of smallpox is 12-13 days, with a range of 9-21 days.[18,19] Exposure in this study was dated from the onset of rash of the index case, since the first day of rash or the day preceding rash is generally considered to mark the onset of infectivity of the case.[18-20] Thus the first six days of exposure would correspond roughly to the first half of the earliest possible presumed incubation period of the exposed contacts, and the next six days to the second half of the incubation period. Among persons treated with methisazone, there was a slightly lower attack rate in those treated during the first six days after exposure, but the difference was not statistically significant and a similar trend was noted in the placebo group.

Effects of vaccination on attack rates. A majority of subjects in Study 1 and 2 and essentially all subjects in Study 3 were vaccinated or revaccinated during the current outbreaks. However, differences in pre- and post-exposure vaccination status allow further analysis of the efficacy of vaccination.

If the placebo group is considered alone, the attack rate in persons unvaccinated prior to exposure was 45.5 per cent. (10/22), as compared to 1.3 per cent. (3/238) in persons previously vaccinated (Table 4). This represents a vaccine-protection ratio of 97.2 per cent.

Among persons unvaccinated prior to exposure, the present study failed to show a difference in overall attack rates between those vaccinated and those not vaccinated after exposure (Table 7); however, the numbers were small and the exact interval between exposure and vaccination was in many instances not known.

The methods of recording data in the earlier studies preclude the calculation of attack rates for persons revaccinated after exposure. However, among previously vaccinated persons (Table 7), the attack rate was significantly lower (0.7 per cent.) for those who had had recent vaccination, i.e. vaccination after exposure or within the past year, than for those who did not have such vaccination (4.6 per cent.).

It has already been shown that no difference in overall attack rates was found between previously vaccinated persons in the methisazone and placebo groups. Similarly, there was no significant difference between the two groups when persons with and without post-exposure or other recent vaccination were examined separately.

Cases. The seven cases which occurred in the methisazone group are described epidemiologicall in Table 8. All were males, and all were 10 years of age or less except for one 18-year-old boy. One of the seven had had pre- and post-exposure vaccination, two had pre-exposure vaccination only, two had post-exposure vaccination only, one had unsuccessful post-exposure primary vaccination and one had no vaccination at all.

The duration of exposure of these contacts to their index cases prior to administration of drug ranged from two to nine days. Of greater interest are the intervals between drug administration and onset of illness, since these indicate the time in the incubation period at which drug was given. As previously noted, one contact was febrile when methisazone was given and developed rash within less than 24 hours. The onset of illness of the other six contacts occurred one, three, eight, nine, 12 and 12 days after drug administration. The four contacts who received drug between one and nine days before onset of illness must have been treated during the incubation period, without effect. The two contacts who became ill 12 days after drug administration may conceivably have been infected after the drug had been excreted or inactivated, assuming that infection occurred very soon after the drug was given or that the incubation period was unusually short. Thus in one of the seven cases methisazone may have been given too late, four cases represent clear-cut drug failures and two cases are possible drug failures.

In addition to the overt cases noted, two contacts reported fever which may have represented variola sine eruptione. One was a 35-year-old woman and one a six-year-old girl. Both had been vaccinated in the past and were revaccinated during the current outbreaks. Each had two days of fever, beginning 12 days after first exposure in one and 17 days after first exposure in the other. One had received methisazone and the other placebo.

Baseline serum specimens were obtained from six of the contacts who subsequently developed smallpox, three in the methisazone group and three in the placebo group. Results of antibody determinations are presented in Table 9. Two of these six contacts had vaccination scars; the four others had no scars, although three of them gave a positive history of vaccination. Most showed negative results in most tests, although all had low levels of neutralizing antibody. In our laboratory, an NT titre of 1:40 or less would be considered non-specific. Two contacts in the methisazone group, one with vaccination scars and one with a history of vaccination but without scar, had neutralizing antibody detectable at dilutions of 1:80 and 1:160, respectively; and these were the only two of the six who had demonstrable CF antibody. These findings presumably represent residual antibody, which failed to provide protection. A similar case has previously been reported by Downie in which a nurse developed a mild attack of smallpox despite the finding of a "considerable titre of neutralizing antibody" in a serum specimen collected immediately prior to infection.[21]

Effect of methisazone on response to vaccination. Serial blood specimens were obtained from 13 contacts in the methisazone group and 12 contacts in the placebo group who had not been vaccinated for at least a year prior to the study, who consumed and retained a full dose of placebo and who were vaccinated by the investigators at the time of drug administration. All subjects who fulfilled these criteria had been vaccinated at some time in the past.

Results of serum antibody determinations are summarized in Table 10. Three contacts in the methisazone group and three in the placebo group had had only primary vaccination prior to the study. All showed fourfold or greater rises in titre in at least two of the four tests, and all three methisazone-treated contacts showed fourfold or greater rises in every test. Ten persons in the drug group and nine in the placebo group had also been revaccinated at some time in the past. As expected, the serologic response of these subjects was in general less marked. However, the majority showed fourfold or greater rises in at least two tests, and there was essentially no difference in response between the methisazone and placebo groups.

Vomiting. Recording of vomiting after drug administration was incomplete in Studies 1 and 2, but in Study 3, the occurrence or non-occurrence of vomiting was specifically recorded in the individual contact questionnaire. During the period of Study 3, 11 out of 106 contacts (10.4 per cent.) in the methisazone group vomited at some time after drug administration, including one who had had less than a full dose. Only one person vomited among the 103 contacts in the placebo group.

DISCUSSION

A trial of a prophylactic agent against smallpox should ideally be carried out without concurrent vaccination, in order that the independent effect of the drug may be assessed. In practice, however, ethical considerations require that vaccination be offered to exposed contacts, either by the investigators or by public health authorities. Thus all the trials of methisazone that have been conducted represent to a large extent tests of methisazone and post-exposure vaccination combined, although most contain smaller subgroups of persons who for various reasons were not vaccinated.

Comparison of studies. The largest trial of methisazone to date was that of Bauer and associates[9,10] in Madras in 1963. In a study group of 5170, they found attack rates of 0.69 per cent. (18/2610) in treated and 3.99 per cent. (102/2560) in untreated contacts. Methisazone was associated with significant reductions in attack rates in persons vaccinated and unvaccinated prior to exposure, whether or not they were vaccinated after exposure. Rao and his associates[12] carried out another study in Madras in 1965-67, limited to unvaccinated contacts. Attack rates were 11.8 per cent. (2/17) in the treated group and 40 per cent. (8/20) in the untreated; the difference was not statistically significant, but the sample was very small.

A trial of methisazone against variola minor was conducted by do Valle et al.[11] in Brazil in 1964-65. This study also showed significant reductions of case incidence by methisazone; 2.1 per cent. of treated contacts (8/384) and 8.1 per cent. of controls (42/520) developed disease.

A study by Ferguson[22] in South Africa is often cited as another trial of methisazone. Four cases occurred among 43 drug-treated contacts. However, since there was not a comparable control group, these can only be regarded as individual case reports of drug failure.

In the present study, overall attack rates were 2.7 per cent. in the drug-treated group (7/262) and 5.0 per cent. in the placebo group (13/260), a difference that was not statistically significant. There was a suggestion of greater effectiveness among contacts unvaccinated prior to exposure.

Since so few trials of methisazone have been conducted and since the logistics of such trials make it unlikely that any further large-scale study will be undertaken in the near future, it is important that an attempt be made to analyse the reasons for differences in results of this and previous studies. Three factors appear relevant: study design, sample size and dosage of drug.

The two largest studies and those which showed the most promising results, the field trials of Bauer and do Valle, were not double-blind and in fact used no placebo in the control group. Treatment was not randomized by individual contact but only by household, and in Bauer's study, variable criteria were used for assignment of contacts to drug and control groups. In the present study and in Rao's treatment was randomized by individual contact, through prior establishment of a randomized schedule of drug and placebo administration. Rao's study and our Study 3 were also double-blind.

It is unlikely that variations in study design would affect the essential results of the trials. On the other hand, the lack of double-blind procedures and predetermined protocol opens possibilities of unconscious bias in admission of subjects, administration of drug and follow-up, which may at least quantitatively affect the results.

Bauer, do Valle and Rao included in their studies only contacts of hospitalized index cases. Thus contacts were isolated from their index cases prior to drug administration and were not at risk of subsequent infection from the same source. In the present study, index cases were in most instances not hospitalized and contacts had continued exposure after

receiving drug. This procedure had the theoretical disadvantage that infection of contacts might have taken place after the drug had been excreted or inactivated. However, as noted above, intervals between drug administration and onset of illness demonstrate that in only two of the seven methisazone-treated cases was it possible that infection might have occurred after drug administration.

Another factor to be considered is sample size. The total numbers of contacts in both treated and control groups were 5170 in Bauer's study, 904 in do Valle's, 37 in Rao's and 522 in the present investigation. Except in the case of Rao's study, the numbers are sufficient to show any clear-cut drug effect. In the present study, it could be argued that a statistically significant drug effect might have been detected if a larger sample had been used. However, even if the sample size were doubled and the proportions were assumed to remain unchanged, the protective effect demonstrated by the drug would be of only borderline significance ($p = .05$). Furthermore, if the results of the double-blind Study 3 were similarly projected, no significant difference would be found between drug and placebo.

Drug dosages used in the various trials must also be considered. Bauer and associates used regimens of a single dose of 3 g, two doses of 3 g, 3 g twice daily for four days and 1.5 g twice daily for four days. They found a significant degree of protection with a single dose of 3 g, which was increased by multiple doses. Similarly, do Valle found a significant protective effect both with a single dose of 3 g and with two doses of 3 g. Rao and associates followed a schedule of 5 g daily for three days for adults, with reduced amounts for children. In the present study, adult regimens of a single dose of 6 g and two doses of 3 g were used, with reduced doses for children under 15. Most of the cases occurred in children. However, five of the seven cases in the methisazone group had received at least as large a dose as the minimum adult dose administered by Bauer and do Valle; the other two, who were one and four years of age, each received 2 g. Thus differences in dosage regimens of the various trials do not appear to be of a nature that would significantly affect the results.

Since all four studies have shown reductions in attack rates in the methisazone-treated groups, it would seem reasonable to conclude that the drug exerts a protective effect. However, the effect is not striking, and there have been substantial numbers of drug failures in each study. Furthermore for the reasons discussed above, even the observed effect may be less than the earlier studies indicated.

Modification of disease. One would expect that an effective antiviral agent would not only reduce case incidence but would also cause modification of disease and reduction of case-fatality ratios. In fact, earlier laboratory studies of methisazone and other thiosemicarbazones showed not only a protective effect but also a prolonged survival time in mice that eventually died.[2,8] No such modifying effect has been found in any of the field trials to date. Bauer's study showed case-fatality rates of 22.2 per cent. (4/18) and 14.7 per cent. (18/102), respectively, in methisazone-treated and control groups; in Rao's study there was one death out of two cases in the drug group and two deaths out of eight cases in the control group; and in the present study, case-fatality ratios were 14.3 per cent. (1/7) and 15.4 per cent. (2/13) in the drug and placebo groups. No deaths occurred in do Valle's study of variola minor, a disease which is rarely fatal. In our study, there was also essentially no difference between the two groups in severity of illness.

The failure of methisazone to modify disease or reduce fatalities may be an indication of inherent ineffectiveness. On the other hand, it may indicate that the drug has an all-or-nothing action in man, perhaps determined by a threshold level of virus and/or drug dosage.

Vomiting. In Bauer's study, the incidence of vomiting ranged from 16.7 to 27.3 per cent. with different dosage regimens, while do Valle found an incidence of 66 per cent. In our Study 3, 10.4 per cent. vomited among 106 contacts who received drug. Despite this variation in rates, it is obvious that at the dosages used in these studies, a substantial number of persons treated would in fact not be afforded the benefit of the drug.

Effect of drug on response to vaccination. The effect of methisazone on the response to vaccination is also of practical importance, since in most situations it would be desirable to vaccinate and administer drug simultaneously. If the drug exerts a suppressive effect on the immunologic response, this factor would have to be considered in deciding for or against its use.

Bauer compared vaccination take rates in contacts in drug and control groups. He found the drug to be associated with a slight reduction in the proportion of successful primary vaccinations and a significant reduction of successful revaccinations. However, in view of the great variability in interpretation of cutaneous reactions[23] and the frequent lack of correlation between the cutaneous and serologic responses[24-26] revaccination take rates can provide only a crude measure of immunologic response.

Landsman & Grist[27] conducted a study in volunteers of the effect of methisazone on the serologic response to vaccination. The amount of drug taken varied from one dose of 3 g only to one dose of 3 g twice daily for three days. Three volunteers who were given primary vaccination and methisazone all showed fourfold or greater rises in haemagglutination-inhibiting antibody titres. However, among subjects who received revaccination, 10 of 16 in the control group but only 4 of 15 in the drug group showed fourfold or greater rises, suggesting a suppressive effect of methisazone.

No such effect was demonstrated in the present study. The serologic response to revaccination was essentially the same in drug and placebo groups, whether the subjects had a history of primary vaccination only or of primary and revaccination. Furthermore, both in our study and in that of Landsman & Grist, the lack of suppressive drug effect was most clear-cut in the least well-immunized groups, in whom suppression would be most dangerous. Three volunteers of Landsman & Grist who received drug at the time of primary vaccination all showed fourfold or greater rises in HI titre. In our study, the three contacts with a history of only primary vaccination all showed at least fourfold rises in all tests. This would suggest that the suppressive drug effect, if any, would be more apt to occur in persons with significant levels of residual antibody, in whom a marked serologic response would in any event be less likely.

Studies of the action of amantadine hydrochloride against experimentally induced influenza have shown that amantadine, while significantly reducing the occurrence and severity of induced A2 influenza, also significantly reduced the mean rise in neutralizing antibody titre after infection.[28] However, the mechanisms of action of the two drugs are not the same. Amantadine acts by inhibiting virus penetration into the cell,[28] while methisazone appears not to prevent cell penetration but in some way inhibits intracellular virus replication.[7] It thus is possible that the action of methisazone, occurring at a later stage, may have considerably less effect than amantadine in reducing the antigenic stimulation.

Efficacy of vaccination after exposure. Finally, the effectiveness of methisazone must be related to that of vaccination, the only prophylactic measure heretofore effective against smallpox.

The high efficacy of vaccination prior to exposure has been consistently demonstrated. Most epidemiologic studies show vaccine-protection ratios of more than 95 per cent., with an even greater degree of protection in persons with a history of revaccination. In a study of intrafamilial transmission among 1249 contacts of smallpox cases, Rao and co-workers found vaccine-protection ratios of 96.2 per cent. in persons with primary vaccination only and 98.9 per cent. in persons who had also had revaccination at some time prior to exposure.[29] The vaccine-protection ratio in the placebo group of the present study was 97.2 per cent.

It was long assumed, on the basis of clinical observations, that post-exposure primary vaccination would similarly afford almost complete protection, if given within the first few days after exposure. On the other hand, there have been many cases reported in which illness occurred despite successful vaccination as early as 12 or 13 days before onset of fever, thus demonstrating that the protection is not complete.[18,19,30] It is very unlikely that a

controlled study of the efficacy of post-exposure primary vaccination will ever be conducted, since a prophylactic procedure of almost certain value cannot be withheld from exposed contacts. However, retrospective studies of outbreaks provide strong evidence that primary vaccination normally affords a high degree of protection if given within the first days after exposure, and that the exceptions are infrequent.

It is similarly difficult to assess the degree of protection provided by revaccination after exposure. However, since the immunologic response to revaccination, as demonstrated both in cutaneous and serologic reactions, occurs several days earlier than after primary vaccination,[19,24] it is logical to assume that revaccination may be effective even if given considerably later in the incubation period.

Conclusions. The results of the present study failed to show a significant difference between the effects of methisazone and placebo. However, since all controlled studies to date, including the present one, have shown some reduction in attack rates in drug-treated groups, it seems probable that methisazone does exert a protective effect, although perhaps less than the results of earlier studies would indicate. None of the studies has demonstrated a modification of disease or reduction of fatalities.

It seems clear that revaccination must remain the prophylactic treatment of choice for exposed contacts of smallpox cases, whether or not they have been vaccinated prior to exposure. It is true that primary vaccination will be ineffective unless given early after exposure, and that failures may occur. However, since one cannot predict in individual cases how soon infection may take place, an attempt at vaccination must be made regardless of the interval since exposure.

Methisazone may provide protection later in the incubation period, by inhibition of virus multiplication, at a time when vaccination would have no effect. It might therefore be recommended as concurrent therapy of possible benefit. It is still unclear whether or not the drug exerts any suppressive effect on the immunologic response to vaccination. However, the available evidence suggests that any such effect would not be sufficient to contra-indicate administration of the drug at the time of vaccination.

## REFERENCES

1. Hamre, D., Bernstein, J. & Donovick, R. (1950) Activity of p-aminobenzaldehyde, 3-thiosemicarbazone on vaccinia virus in the chick embryo and in the mouse, Proc. Soc. exp. Biol. Med., 73, 275-278

2. Thompson, R. L., Price, M. L. & Minton, S. A. (1951) Protection of mice against vaccinia virus by administration of benzaldehyde thiosemicarbazone., Proc. Soc. exp. Biol. Med., 78, 11-13

3. Minton, S. A.,jr., Officer, J. E. & Thompson, R. L. (1953) Effect of thiosemicarbazones and dichlorophenoxy thiouracil on multiplication of a recently isolated strain of variola-vaccinia virus in the brain of the mouse, J. Immunol., 70, 222-228

4. Thompson, R. L. et al. (1953) Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse, J. Immunol., 70, 229-234

5. Bauer, D. J. (1955) The antiviral and synergic actions of isatin thiosemicarbazone and certain phenoxypyrimidines in vaccinia infection in mice, Brit. J. exp. Path., 36, 105-114

6. Bauer, D. J. & Sadler, P. W. (1960) The structure-activity relationships of the anti-viral chemotherapeutic activity of isatin-thiosemicarbazone, Brit. J. Pharmacol., 15, 101-110

7. Bach, M. K. & Magee, W. E. (1962) Biochemical effects of isatin-thiosemicarbazone on development of vaccinia virus, Proc. Soc. exp. Biol. Med., 110, 565-567

8. Bauer, D. J. et al. (1962) The chemotherapy of variola major infection, Bull. Wld Hlth Org., 26, 727-732

9. Bauer, D. J. et al. (1963) Prophylactic treatment of smallpox contacts with 1-methy-lisatin-thiosemicarbazone, Lancet, 2, 494-496

10. Bauer, D. J. et al. (1969) Prophylaxis of smallpox with methisazone, Amer. J. Epid., 90, 130-145

11. do Valle, L. A. R. et al. (1965) Methisazone in prevention of variola minor among contacts, Lancet, 2, 976-978

12. Rao, A. R. et al. (1969) Chemoprophylaxis and chemotherapy in variola major. I. An assessment of CG 662 and Marboran in prophylaxis of contacts of variola major, Indian J. Med. Res., 57, 477-483

13. Kempe, C. H. & St. Vincent, L. (1964) Variola and vaccinia viruses. In: Lennette, E. H. & Schmidt, N. J., eds, Diagnostic Procedures for Viral and Rickettsial Diseases, 3rd ed. Amer. Public Health Assoc., N.Y., N.Y., pp. 665-692

14. Anthony, R. L. et al. Studies of variola virus and immunity in smallpox. II. Recovery of virus-specific soluble antigens from infected Rhesus monkey kidney cells, J. Infect. Dis. (In press)

15. Anthony, R. L. et al. (1970) Studies of variola virus and immunity in smallpox. I. Variola virus hemagglutinins, J. Infect. Dis., 121, 295-302

16. Anthony, R. L. et al. Studies of variola virus and immunity in smallpox. III. Measurement of antibodies to high molecular weight soluble antigens of variola virus using the bis-diazotized benzidine passive hemagglutination technique. (Submitted for publication)

17. Cutchins, E., Warren, J. & Jones, W. P. (1960) The antibody response to smallpox vaccination as measured by a tissue culture plaque method, J. Immunol., 85, 275-283

18. Dixon, C. W. (1962) Smallpox, J. & A. Churchill Ltd., London

19. Downie, A. W. (1965) Poxvirus group. In: Horsfall, F. L. jr. & Tamm, I. eds., Viral and Rickettsial Infections of Man, 4th ed., Philadelphia, J. B. Lippincott Co., pp. 932-964

20. Downie, A. W. et al. (1961) Studies on the virus content of mouth washings in the acute phase of smallpox, Bull. Wld Hlth Org., 25, 49-53

21. Downie, A. W. (1951) Infection and immunity in smallpox, Lancet, 1, 419-422

22. Ferguson, D. L. (1964) Some observations on the role of methisazone ('Marboran') in the prophylaxis of smallpox in a rural area, S. Afr. Med. J., 38, 868-869

23. Benenson, A. S. (1950) Immediate (so-called "immune") reaction to smallpox vaccination, J.A.M.A., 143, 1238-1240

24. McCarthy, K., Downie, A. W. & Bradley, W. H. (1958) The antibody response in man following infection with viruses of the pox group. II. Antibody response following vaccination, J. Hyg., Camb., 56, 466-478

25. Collier, W. A. (1951) Individual immunity against smallpox, Documenta Nederlandica et Indonesica de Morbis Tropicis, 3, 163-176

26. Nyerges, G., Hollos, I. & Barsy, G. (1966) The significance of serological tests in controlling the success of smallpox revaccination, Acta Microbiol, Acad. Sci. Hung., 13, 97-112

27. Landsman, J. B. & Grist, N. R. (1964) Controlled trial of Marboran on group vaccinated against smallpox, Lancet, 1, 330

28. Togo, Y., Hornick, R. B. & Dawkins, A. T., jr (1968) Studies on induced influenza in man. I. Double-blind studies designed to assess prophylactic efficacy of amantadine hydrochloride against A2/Rockville/1/65 strain, J.A.M.A., 203, 1089-1094

29. Rao, A. R. et al. (1968) Epidemiological studies in smallpox. A study of intrafamilial transmission in a series of 254 infected families, Ind. J. Med. Res., 56, 1826-1854

30. Marsden, J. P. (1948) Variola minor. A personal analysis of 13 686 cases, Bull. Hyg., 23, 735-746

TABLE 1. DISTRIBUTION OF CONTACTS AND OF SUBSEQUENT CASES BY TREATMENT CATEGORY

| Treatment category | Study 1 (1964) | | Study 2 (1965) | | Study 3 (1966-70) | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Contacts | Cases | Contacts | Cases | Contacts | Cases | Contacts | Cases | Deaths | Attack rate (%) |
| Methisazone | | | | | | | | | | |
| Full dose | 38 | 1 | 103 | 2 | 88 | 3 | 229 | 6 | 1 | 2.6 |
| Incomplete dose | 4 | 0 | 1 | 0 | 8 | 0 | 13 | 0 | 0 | 0.0 |
| Vomited* | 3 | 0 | 7 | 0 | 10 | 1 | 20 | 1 | 0 | 5.0 |
| Total methisazone | 45 | 1 | 111 | 2 | 106 | 4 | 262 | 7 | 1 | 2.7 |
| Placebo | 51 | 5 | 106 | 3 | 103 | 5 | 260 | 13 | 2 | 5.0 |
| Other untreated | | | | | | | | | | |
| Refused | 1 | 1 | 3 | 0 | 22 | 0 | 26 | 1 | 0 | 3.8 |
| Absent | 0 | 0 | 0 | 0 | 22+ | 0 | 22+ | 0 | 0 | 0.0 |
| Total untreated | 52 | 6 | 109 | 3 | 147 | 5 | 308 | 14 | 2 | 4.5 |
| Total | 97 | 7 | 220 | 5 | 253 | 9 | 570 | 21 | 3 | 3.7 |

*Not including 3 contacts who had incomplete dose and also vomited.

+Including 9 contacts who were excluded by error

TABLE 2. DISTRIBUTION OF CONTACTS IN METHISAZONE AND PLACEBO GROUPS BY AGE AND SEX

| Age (years) | Methisazone | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | Females | Both sexes | % of total | Males | Females | Both sexes | % of total |
| 0 - 4 | 16 | 13 | 29 | 11.1 | 20 | 20 | 40 | 15.4 |
| 5 - 9 | 30 | 17 | 47 | 17.9 | 33 | 27 | 60 | 23.1 |
| 10 - 14 | 25 | 23 | 48 | 18.3 | 18 | 14 | 32 | 12.3 |
| 15 - 19 | 10 | 12 | 22 | 8.4 | 13 | 14 | 27 | 10.4 |
| ≥ 20 | 55 | 58 | 113 | 43.1 | 50 | 51 | 101 | 38.8 |
| Unknown | 1 | 2 | 3 | 1.1 | 0 | 0 | 0 | 0.0 |
| All ages | 137 | 125 | 262 | 100.0 | 134 | 126 | 260 | 100.0 |
| % of total | 52.3 | 47.7 | 100.0 | | 51.5 | 48.5 | 100.0 | |

TABLE 3. DISTRIBUTION OF CONTACTS IN METHISAZONE AND PLACEBO GROUPS BY PRE-EXPOSURE VACCINATION STATUS

| | Methisazone | | Placebo | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Unvaccinated | 18* | 6.9 | 22+ | 8.5 |
| Vaccinated | 244 | 93.1 | 238 | 91.5 |
| Total | 262 | 100.0 | 260 | 100.0 |

* Including 2 persons with history of vaccination but without scar

\+ Including 5 persons with history of vaccination but without scar

TABLE 4. ATTACK RATES IN CONTACTS BY PRE-EXPOSURE VACCINATION STATUS AND TREATMENT CATEGORY

| Treatment category | Attack rate (per cent) | | |
|---|---|---|---|
| | Unvaccinated | Vaccinated | Total |
| Methisazone | 22.2 (4/18) | 1.2 (3/244) | 2.7 (7/262) |
| Placebo | 45.5 (10/22) | 1.3 (3/238) | 5.0 (13/260) |
| Total | 35.0 (14/40) | 1.2 (6/482) | 3.8 (20/522) |

TABLE 5. ATTACK RATES IN CONTACTS BY PRE-EXPOSURE VACCINATION STATUS AND METHISAZONE DOSAGE REGIMEN

| Methisazone dosage | Attack rate (per cent) | | |
|---|---|---|---|
| | Unvaccinated | Vaccinated | Total |
| 6g | 23.1 (3/13) | 1.5 (3/204) | 2.8 (6/217) |
| 2 x 3g | 20.0 (1/5) | 0.0 (0/40) | 2.2 (1/45) |
| Total methisazone | 22.2 (4/18) | 1.2 (3/244) | 2.7 (7/262) |

TABLE 6. ATTACK RATES IN CONTACTS BY PRE-EXPOSURE VACCINATION STATUS, DURATION OF EXPOSURE AT TIME OF DRUG ADMINISTRATION AND TREATMENT CATEGORY

| Duration of exposure | Treatment category | Attack rate (per cent) | | |
|---|---|---|---|---|
| | | Unvaccinated | Vaccinated | Total |
| ≤ 6 days | Methisazone | 20.0 (2/10) | 1.3 (2/154) | 2.4 (4/164) |
| | Placebo | 33.3 (4/12) | 1.3 (2/155) | 3.6 (6/167) |
| | Total | 27.3 (6/22) | 1.3 (4/309) | 3.0 (10/331) |
| > 6 days | Methisazone | 25.0 (2/8) | 1.1 (1/90) | 3.1 (3/98) |
| | Placebo | 60.0 (6/10) | 1.2 (1/83) | 7.5 (7/93) |
| | Total | 44.4 (8/18) | 1.2 (2/173) | 5.2 (10/191) |

TABLE 7. ATTACK RATES IN CONTACTS BY PRE-EXPOSURE VACCINATION STATUS, RECENT OR POST-EXPOSURE VACCINATION AND TREATMENT CATEGORY

| Treatment category | Attack rates (per cent) | | | | | |
|---|---|---|---|---|---|---|
| | Methisazone | | Placebo | | Total | |
| Unvaccinated prior to exposure | | | | | | |
| Not vaccinated after exposure | 100.0 | (1/1) | 33.3 | (1/3) | 50.0 | (2/4) |
| Vaccinated after exposure | 17.6 | (3/17) | 47.4 | (9/19) | 33.3 | (12/36) |
| Vaccinated at time of drug administration* | 40.0 | (2/5) | 50.0 | (5/10) | 46.7 | (7/15) |
| Vaccinated prior to exposure | | | | | | |
| Not vaccinated after exposure or within past year | 5.7 | (2/35) | 3.3 | (1/30) | 4.6 | (3/65) |
| Vaccinated after exposure or within past year | 0.5 | (1/209) | 1.0 | (2/208) | 0.7 | (3/417) |
| Vaccinated at time of drug administration* | 2.9 | (1/35) | 0.0 | (0/28) | 1.6 | (1/63) |

* Excluding persons already vaccinated after exposure or within past year

TABLE 8. CASES OF SMALLPOX IN METHISAZONE-TREATED GROUP

| Study no. | Case | Age (years) and sex | Vaccination | | Drug | | Intervals (days) | | | | | Course of illness |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Before exposure* | After exposure | Dosage | Adult equivalent | Exposure to vaccination | Exposure to day of drug | Exposure to onset of illness | Vaccination to onset of illness | Day of drug to onset of illness | |
| 1 | M.J. | 11 mo.M | 0 | 0+ | 1½g x 2 | 3g x 2 | 3 | 5 | ‡ | + | Already febrile | Died |
| 2 | S.D. | 3 M | 0 | 0 | 3g | 6g | - | 3 | 15 | - | 12 | Moderate |
| 2 | I. | 2 M | + | 0 | 3g | 6g | - | 2 | 11 | - | 9 | Severe |
| 3 | Y. | 4 M | 0** | + | 2g | 6g | 4 | 4 | 12 | 8 | 8 | Mild |
| 3 | A. | 18 M | + | 0 | 6g | 6g | - | 5 | 17 | - | 12 | Moderate |
| 3 | M. | 10 M | + | + | 4g | 6g | 9 | 9 | 10 | 1 | 1 | Moderate |
| 3 | A. | 1 M | 0 | + | 2g | 6g | 7 | 7 | 10 | 3 | 3 | Moderate |

* Vaccination scar present
** Positive history of vaccination but no detectable scar
\+ Received unsuccessful primary vaccination after exposure
‡ Febrile at time of first visit, 5 days after onset of rash of index case, and presumed to be a co-primary case.

TABLE 9. ANTIBODY DETERMINATIONS IN SERA OF CONTACTS WHO SUBSEQUENTLY DEVELOPED SMALLPOX

| Case | Age and sex | Pre-exposure vaccination* | Drug (M) or placebo (P) | Serologic results | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | No. days before onset+ | Reciprocal of titre | | | |
| | | | | | CF | HI | PHA | NT |
| Y. | 4 M | 0** | M | 8 | 10 | <4++ | <10 | 160 |
| A. | 18 M | + | M | 12 | 10 | <4 | <10 | 80 |
| M. | 10 M | + | M | 1 | <10 | <4 | <10 | 20 |
| M. | 8 M | 0** | P | 3 | <10 | ‡ | ‡ | <100 |
| F.B. | 28 F | 0 | P | 13 | <10 | <4 | <10 | 10 |
| A. | 6 M | 0** | P | 6 | <10 | <4 | 20 | 40 |
| International Standard Anti-Smallpox Serum | | | | | 160 | 128 | 320 | 10,240 |

* Vaccination scar present
** Positive history of vaccination but no detectable scar
\+ Onset of illness, defined as first day of fever
++ Filter-paper specimen, at initial dilution of 1:5
‡ Insufficient volume

TABLE 10. SERIAL ANTIBODY DETERMINATIONS IN SERA OF CONTACTS WHO RECEIVED REVACCINATION AND SIMULTANEOUS ADMINISTRATION OF METHISAZONE OR PLACEBO

| | Contacts with history of primary vaccination only | | Contacts with history of primary and revaccination | |
|---|---|---|---|---|
| | Methisazone | Placebo | Methisazone | Placebo |
| No. of contacts | 3 | 3 | 10 | 9 |
| Interval between specimens 1 and 2 | | | | |
| Range (days) | 49 | 40 - 57 | 42 - 63 | 41 - 66 |
| Mean (days) | 49 | 48.7 | 50.8 | 47.1 |
| Geometric mean titre (reciprocal) | | | | |
| Specimen 1: CF | < 10 | 10 | < 10 | < 10 |
| HI | < 4 | 4 | 4.3 | < 4 |
| PHA | < 10 | < 10 | 18.6 | 12.6 |
| NT | 40.0 | 40.0 | 196.8 | 63.6 |
| Specimen 2: CF | 100.8 | 40.0 | 13.2 | 20.0 |
| HI | 16.0 | 32.0 | 10.6 | 6.8 |
| PHA | 3,225.6 | 640.0 | 211.2 | 117.6 |
| NT | 2,560.0 | 640.0 | 966.4 | 870.4 |
| Mean multiple of rise in titre | | | | |
| CF | 21.3 | 5.3 | 5.8 | 7.1 |
| HI | 9.3 | 14.0 | 5.0 | 3.3 |
| PHA | 426.7 | 373.3 | 59.4 | 21.6 |
| NT | 90.7 | 20.0 | 10.6 | 45.8 |
| % of sera showing fourfold or greater rise in titre | | | | |
| CF | 100.0 (3/3) | 66.7 (2/3) | 30.0 (3/10) | 55.6 (5/9) |
| HI | 100.0 (3/3) | 66.7 (2/3) | 50.0 (5/10) | 44.4 (4/9) |
| PHA | 100.0 (3/3) | 100.0 (3/3) | 60.0 (6/10) | 77.8 (7/9) |
| NT | 100.0 (3/3) | 100.0 (2/2) | 50.0 (5/10) | 77.8 (7/9) |