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## Toxicity of Hydroxamic Acid Analogues; Prophylactic and Therapeutic Efficacy Against Nerve Gas Poisoning in Mice.* (22575)

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Hydroxylamine HCl and a series of its analogues were shown to accelerate the hydrolysis of phosphoric acid esters by splitting off the phosphate groups(1). Some of the hydroxamic acid derivatives (HA) are capable also of reactivating cholinesterase that had been inhibited by reaction with phosphoric acid esters, such as nerve gases and certain of the insecticides. This is accomplished by releasing the phosphate bound in the reaction, thus freeing the enzyme(2-6).

These evidences of potential prophylactic and therapeutic use of HA compounds were tested in mice intoxicated with nerve gas or with phosphate insecticides(7). Only studies with the nerve gas, Sarin (isopropyl-methyl-phosphono-fluoride), are reported here.

*Method.* Hydroxylamine and analogues of hydroxamic acid (HA) were tested both for their individual toxicities and for their efficacies in reducing the toxicity of Sarin in albino mice. Each HA was made up as a solution in 0.85% NaCl, neutralized and adjusted so that 1 ml contained approximately one $LD_{50}$ when administered intraperitoneally. For each analogue groups of 6 to 8 mice were injected with 4 to 6 different doses covering a range of approximately the interval $LD_1$-$LD_{100}$. The $LD_{50}$ for each analogue was calculated by the method of Litchfield and Wilcoxon(8). These are listed in Table I. Mice generally died within one hour. A few succumbed during the second hour and those that survived for 2 hours seldom died during the subsequent 18 hours of observation. Only deaths within 2 hours were used in the calculation of the $LD_{50}$. Animals were observed

TABLE I. Approximate $LD_{50}$'s in Mice of An Analogous Series of Hydroxamates Injected Intraperitoneally.

| Hydroxamate | Approximate $LD_{50}$ (mg/g) |
|---|---|
| Hydroxylamine | .15 |
| Salicyl- | .15 |
| Nicotin- (benzobromide) | .16* |
| Polyacryl- | .16 |
| Diglycolic di- | .17* |
| Picolin- | .3 |
| Tartar- | .5 |
| Sorb- | .7 |
| Glycin- | .8 |
| Acet- | 1.3 |
| Nicotin- | 2.3 |
| n-dimethyl glycin- (methochloride) | 2.5 * |
| Glucuron- | 3.0 |
| Histidin- | 3.7 |
| Nicotin- (methiodide) | 4.0 |
| Serin- | 6.5 |
| Lact- | 8.4 |
| Mandel- | >5.0 † |

* Tested against 2 × $LD_{50}$ only (Table II).
† Limited by solubility.

* Synthesis of hydroxamates by Drs. T. Wagner-Jauregg, G. M. Steinberg and R. Plapinger, Medical Laboratories, Army Chemical Center, Md. Statistical analysis was done by Mr. I. A. DeArman, Medical Laboratories, Army Chemical Center, Md.

TABLE II. Effectiveness of Hydroxamic Acid Analogues, Given Intraperitoneally, against One or Two $LD_{50}$'s of Sarin Given Subcutaneously in Mice.

| Hydroxamate | Min. from nerve gas inj. | Dose, mg/g | Potency ratio† | No. dead /No. inj.‡ |
|---|---|---|---|---|
| Glycin- | −15 | .4 | 1.5 | |
| | −10 | .4 | 1.6* | |
| | − 2 | .4 | 2.4* | |
| | 2.5 | .5 | 2.5* | |
| | 10 | .5 | 2.1* | |
| | 12 | .5 | 1.5 | |
| Lact- | − 5 | .6 | 2.8* | |
| | − 5 | .04 | 1.0 | |
| | 5 | 2.0 | 2.8* | |
| | 5 | .4 | .8 | |
| Nicotin- | − 5 | .9 | 2.1* | |
| | − 5 | .5 | 2.2* | |
| | 5 | 1.7 | 2.9* | |
| Nicotin- (methiodide) | − 5 | 2.0 | 2.2* | |
| | − 5 | .4 | 1.2 | |
| | 5 | 2.0 | 2.9* | |
| | 5 | .4 | 1.0 | |
| Picolin- | − 5 | .2 | 1.0 | |
| | 5 | .2 | .7 | |
| | 1-2 | .2 | 1.0 | |
| Serin- | − 5 | 1.5 | 1.4 | |
| | − 5 | 1.5 | 2.7* | |
| Citric- | − 5 | .4 | 1.3 | |
| Sorb- | − 5 | .2 | 1.5 | |
| Polyacryl- | − 5 | .4 | 1.0 | |
| | − 5 | .75 | 1.0 | |
| Glucuron- | − 5 | 1.0 | 1.4 | |
| n-dimethyl glycin- (methochloride) | − 5 | 1.0 | | 8/24 |
| Nicotin- (benzobromide) | − 5 | .8 | | 11/24 |
| Diglycolic di- | − 5 | .8 | | 22/24 |

* Significantly $> 1.0$.
† Calculated $LD_{50}$ compared to that of control mice.
‡ Against $2 \times LD_{50}$ ($> LD_{100}$) Sarin.

also for clinical evidence of toxicity as indicated by appearance and behavior with all doses for the purpose of choosing an initial trial protective dose.

Next the analogues were tested for protective efficacy in mice poisoned subcutaneously with one $LD_{50}$ of Sarin. In order to control possible variability in the toxicity of Sarin the $LD_{50}$ was estimated each time that an HA was to be tested against it. This was done by injecting 4 to 6 groups of 6 mice each with different doses of Sarin(8). On the same day 2 groups of 24 mice each were injected with the $LD_{50}$ of Sarin subcutaneously. Of these groups one was injected intraperitoneally with HA also and the other with saline. The protective doses chosen for each HA used, listed in Table II, range up to ⅔ of the $LD_{50}$ of the HA, the largest doses given being smaller than those provoking signs of toxicity. HA was given either before or after Sarin. (The terms "therapeutic" and "prophylactic" refer only to the time relationship with administration of Sarin rather than to mechanism.) The intervals to or from the time of injection of Sarin are listed in Table II. To assess the efficacy of an HA for each experimental situation, the numbers of dead out of 24 in the 2 groups were examined and the difference tested by means of $\chi^2$ in a 2x2 contingency table(9). This is equivalent to testing the equality of the proportion dead in the 2 groups. The index chosen to measure the relative efficacy of an HA is the potency ratio (10). This was computed by pooling the results from all preliminary assays of Sarin, and computing a slope. It was then assumed that the slopes of the response lines of the experiments with HA would be equal to this common slope for all experiments. Using the common slope and the responses observed in each of the efficacy trials of HA against a relatively standard dose of Sarin, $LD_{50}$'s were estimated for each trial. Potency ratios were computed and appear in Table II. The significance of the potency ratio was determined by the $\chi^2$ test. If the numbers of dead in the 2 groups were significantly different, the ratio was assumed to be significantly different from 1. In addition 3 HA's were tested against twice the $LD_{50}$ of Sarin, a dose that kills all mice, and the results are shown at the end of Table II.

*Results.* Toxicity levels for the analogous series of hydroxamates, administered intraperitoneally, are listed in descending order (Table I) and indicate a wide range among those tested. It is of special interest that the potency ratios shown in Table II appear to be independent of the relative toxicities. For example, lact-hydroxamate is the least toxic and one of the more effective compounds. On the other hand, glycin-hydroxamate is more toxic and quite ineffective. The last 3 compounds listed in Table II protected all mice against

one $LD_{50}$ of Sarin and gave partial protection against 2, a uniformly fatal dose. Thus far, compounds having protective properties when injected several minutes prior to Sarin may also be effective when administered within a few minutes after the toxic material (Table II). "Prophylactic" or early "therapeutic" use of the hydroxamates intraperitoneally appears to be more effective, however, than their use subsequently. When administered after the onset of obvious signs of intoxication, the effects of these hydroxamates were negligible in terms of reversing the developing toxic manifestations in mice.

*Discussion.* Although there are striking hydrolytic effects of hydroxamic acid analogues *in vitro*(1-6), protection requires relatively huge doses in mice. This is of practical importance in application. Factors pertaining to optimum conditions for contact between the antidote and either free Sarin or Sarin-inhibited enzymes or both, *in vivo*, include rates of absorption and excretion, of degradation and detoxification and of diffusion from the circulation. The apparent independence of toxicity and potency ratios among candidate compounds encourages further exploration. It would appear that toxicity of an HA may limit the prospect of testing its maximum efficacy. However, the large doses required to raise the $LD_{50}$ of Sarin significantly would appear to limit the therapeutic potential. Related compounds are being studied.

*Summary.* Some hydroxamic acid analogues protect mice against an $LD_{50}$ dose of Sarin. Effective doses required in this series have been large. The efficacy of hydroxamates is variable and unrelated to toxicity.

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## Host Resistance to Bacteria in Hemorrhagic Shock. VI. Effect of Endotoxin on Antibacterial Defense.* (22576)

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Transfusion for experimental hemorrhagic shock of 2 hours duration is followed by recovery. But if the shock is allowed to persist for much longer (4-5 hours) it fails to respond to transfusion. Evidence has been given that the failure of the circulation to respond to transfusion is a result of bacterial activity, which cannot be controlled because shock produces a progressively severe injury to the antibacterial mechanisms(1-6). The further observation that intravenous antibiotic therapy, given shortly after shock was induced, failed to prevent the development of irreversibility, in contrast to the success of such therapy when given before shock was induced

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