mice, as compared to the untreated controls, was observed(8). No significant modifying effect of either progesterone or relaxin was apparent.

*Summary.* Little or no increase in the percentage of positive mammary alveolar responses was obtained in ovariectomized mice treated with estrogen plus progesterone plus relaxin as compared to those treated with estrogen plus progesterone only. Progesterone inhibited the pelvic relaxing effect of estrogen or of estrogen plus relaxin. Neither progesterone nor relaxin exerted any significant modifying effect on the increased bone density resulting from estrogen administration.

8. Gardner, W. U., and Pfeiffer, C. A., *Physiol. Rev.*, 1943, v23, 139.

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## Protection of Mice Against Vaccinia Virus by Administration of Benzaldehyde Thiosemicarbazone.* (18957)

R. L. THOMPSON, MARIAN L. PRICE, AND S. A. MINTON, Jr.

*From the Department of Microbiology, Indiana University Medical Center, Indianapolis.*

Domagk and associates(1) reported that benzaldehyde thiosemicarbazone inhibited growth of the tuberculosis bacillus *in vitro*. Certain derivatives of this compound were found to protect experimental animals against tuberculosis(2). Hamre *et al.*(3,4) investigated the effect of substituted benzaldehyde thiosemicarbazones on other microorganisms. The *p*-amino analogue was observed to delay death and to protect a small percentage of chick embryos and mice infected with vaccinia virus. The *p*-acetamido analogue was less effective.

The present report deals with the effect of benzaldehyde thiosemicarbazone and of several *para* substituted derivatives on vaccinia virus. The compounds used in the study were obtained from Dr. A. F. Langlykke of E. R. Squibb and Sons.

*Experimental.* In *in vitro* experiments the CVII strain of vaccinia virus was cultivated in a medium containing minced chick embryonic tissues(5). The International Health Division (IHD) strain of virus was employed in *in vivo* tests(6). Mice, in groups of 6, were inoculated with viral dilutions of $10^{-2}$, $10^{-3}$ and $10^{-4}$ by the cerebral route. Materials to be tested were fed to mice in a sucrose-casein diet.

*Results.* Benzaldehyde thiosemicarbazone (No. 1) was found to inhibit completely viral multiplication when present in a concentration of 1 $\mu$g per ml of culture medium (Table I). Substitutions in the *para* position of the benzene nucleus reduced activity in all instances (No. 2-8). The introduction of an isobutyl group in the 4-position of the thiosemicarbazone portion of the molecule (No. 9) likewise decreased *in vitro* activity. With the exception of compound No. 3, none of the substances was completely in solution when added to the culture medium.

The failure of vaccinia virus to multiply in the presence of thiosemicarbazones does not appear to be due to inactivation of the virus by these substances. Benzaldehyde thiosemicarbazone was added to Tyrode's solution containing 5% normal rabbit serum and a known strength of vaccinia virus to give a concentration of 0.05 mg of the compound per ml of

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1. Domagk, G., Behnisch, R., Mietsch, F., and Schmidt, H., *Naturwissensch.*, 1946, v33, 315.

2. Domagk, G., *Beitrage z. Klin. d. Tuber.*, 1948, v101, 365.

3. Hamre, D., Bernstein, J., and Donovick, R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 275.

4. Brownlee, K. A., and Hamre, D., *J. Bact.*, 1951, v61, 127.

5. Thompson, R. L., *J. Immunol.*, 1947, v55, 345.

6. Thompson, R. L., Price, M. L., Minton, S. A., Jr., Falco, E. A., and Hitchings, G. H., *J. Immunol.*, in press.

TABLE I. Effect of Benzaldehyde Thiosemicarbazones on Vaccinia Virus.

| No. | Substituent in *p*-position of benzene ring | Conc. (mg/ml) | Inhibition of viral growth in chick embryonic tissues change in titer of virus (log) In presence of compound | In absence of compound | Protection of mice against virus (Intracerebral) Conc. in diet (%) | Treatment started (day) | Proportion of mice surviving infection Treated | Untreated | Excess survivors in treated groups |
|---|---|---|---|---|---|---|---|---|---|
| | | | Benzaldehyde thiosemicarbazones | | | | | | |
| 1 | — | .1 | −2 * | 1.9 | .02 | −2 | 10/16 | 2/16 | 8 |
| | | .01 | −1.6* | 1.5 | .04 | −2 | 11/17 | 2/16 | 9 |
| | | | | | .08 | −2 | 10/15 | 2/16 | 8 |
| | | .001 | −2.2* | 1.5 | | | | | |
| | | | | | .03 | −3 | 8/18 | 4/18 | 4 |
| | | | | | .04 | −2 | 9/18 | 4/18 | 5 |
| | | | | | .04 | −2 | 13/18 | 2/18 | 11 |
| | | | | | .04 | 0 | 10/18 | 2/18 | 8 |
| | | | | | .04 | 2 | 7/17 | 2/17 | 5 |
| | | | | | .04 | 2 | 6/18 | 2/18 | 4 |
| | | | | | .04 | −2 | 9/18 | 1/17 | 8 |
| | | | | | .04 | −2 | 12/18 | 3/18 | 9 |
| | | | | | .04 | −2 | 9/17 | 1/17 | 8 |
| | | | | | .04 | −1 | 13/18 | 6/18 | 7 |
| | | | | | .03 | −3 | 17/18† | 7/18† | 10 |
| 2 | hydroxy | .05 | − .5 | 1.9 | .04 | −2 | 5/17 | 5/18 | 0 |
| | | | | | .04 | −1 | 6/18 | 3/18 | 3 |
| 3 | amino | .1 | − .7 | 1.9 | .04 | −2 | 4/15 | 2/17 | 2 |
| | | | | | .04 | −1 | 2/17 | 1/17 | 1 |
| | | | | | .04 | 0 | 7/17 | 5/17 | 2 |
| 4 | acetamido | .1 | .1 | 1.9 | .04 | 0 | 5/18 | 1/18 | 4 |
| | | | | | .05 | −2 | 3/17 | 6/18 | −3 |
| | | | | | .06 | −3 | 0/18 | 4/18 | −4 |
| | | | | | .1 | 0 | 2/17 | 1/18 | 1 |
| 5 | dimethylamino | .05 | − .3 | 1 | .04 | −3 | 5/18 | 6/18 | −1 |
| 6 | isopropyl | .05 | −2 * | 1 | .04 | −3 | 1/18 | 3/18 | −2 |
| | | .005 | .7 | 1.3 | | | | | |
| 7 | ethylsulfonyl | .05 | −1.3* | 1.9 | .04 | −1 | 5/17 | 4/18 | 1 |
| | | .01 | 1 | 1.5 | .04 | −1 | 10/18 | 5/18 | 5 |
| | | | | | .06 | −2 | 3/18 | 3/18 | 0 |
| 8 | isobutoxy | .05 | −2.3* | 1.4 | .025 | −2 | 5/18 | 3/18 | 2 |
| | | .005 | −1 | 1.5 | .04 | −2 | 7/18 | 3/18 | 4 |
| | | | | | .04 | −1 | 4/18 | 1/18 | 3 |
| | | | | | .04 | 1 | 5/18 | 2/17 | 3 |
| | | | Benzaldehyde 4-isobutyl thiosemicarbazones | | | | | | |
| 9 | — | .05 | − .5 | 1 | .04 | −3 | 5/18 | 6/18 | −1 |
| 10 | acetamido | .05 | − .5 | 1.4 | .04 | −2 | 2/18 | 3/18 | −1 |
| 11 | dimethylamino | .05 | −1 | 1 | .04 | −3 | 5/18 | 6/18 | −1 |
| 12 | ethylsulfonyl | .05 | 2.3 | 1.9 | .04 | −3 | 6/18 | 6/18 | 0 |

* Or greater.

† Mice were inoculated with the WR strain of virus; in all other experiments the IHD strain was used.

medium. After incubation at 32°C for two days the concentration of virus was found to have decreased 2.3 log units. In corresponding control tests the reduction was 1.9 log units. In a comparable experiment with the *p*-acetamido analogue, using a concentration of 0.1 mg per ml of medium, reductions of 2.8 and 2.2 log units, respectively, were obtained.

In *in vivo* tests, benzaldehyde thiosemicarbazone was found to be more active than any other member of the series (Table I). In 14 experiments in which this compound was fed in the diet in concentrations varying from 0.02 to 0.08%, 144 of 244 treated mice (59%) survived in contrast to 40 of 243 untreated animals (16%). Treated mice which succumbed to infection died on the average of one day later than the corresponding controls. In one experiment benzaldehyde thiosemicarbazone was used for the treatment of mice inoculated intracerebrally with the WR

(Western Reserve) strain of vaccinia virus. In this experiment, 17 of 18 treated mice survived as compared to 7 of 18 untreated animals.

The number of survivors in treated groups exceeded the number in control groups in experiments with *p*-amino (3 of 3), *p*-isobutoxy (4 of 4), *p*-ethylsulfonyl (2 of 3), *p*-hydroxy (1 of 2) and *p*-acetamido (2 of 4) analogues.

*Discussion.* Although benzaldehyde thiosemicarbazone inhibits growth of the tuberculosis bacillus *in vitro*(1,7), it is relatively inactive *in vivo*(8,9). Derivatives of benzaldehyde thiosemicarbazone with ethylsulfonyl, acetamido(2), amino and dimethylamino(8,9) substituents in the *para* position of the benzene nucleus are very effective for the treatment of tuberculosis in experimental animals. These substances, however, have been observed to produce little or no protection against vaccinia infection in mice. In contrast, the unsubstituted compound exhibits a marked degree of protection of mice against vaccinia virus.

The mode of action of the thiosemicarbazones in tuberculosis has not been established. Domagk(10) has expressed the view that the therapeutic effects observed are the result of a direct action of these compounds on the tuberculosis organism and not a consequence of some theoretical indirect action on the host. In the case of vaccinia virus, it was observed that the compounds (No. 1, 8) which were most virustatic *in vitro* were the most effective materials in protection tests. Since the thiosemicarbazones do not appear to inactivate the virus as the result of prolonged contact, it may be assumed that they act, not by a direct virucidal action, but by interference with proliferation of the infectious agent. The inhibition of viral multiplication may be the result either of a direct action by the thiosemicarbazones on the virus or indirectly, possibly by alteration of the metabolic processes of the tissues of the host.

It is of interest to note that certain phenoxythiouracils protect mice against vaccinia virus(6). These compounds, like the thiosemicarbazones, contain a thiourea group. When administered in the diet, the dosage of phenoxythiouracils required to protect mice against vaccinia virus is 10 to 20 times greater than that of thiosemicarbazones. Active members of both groups of compounds are relatively insoluble in distilled water.

*Summary.* Benzaldehyde thiosemicarbazone prevents multiplication of vaccinia virus in chick embryonic tissues when present in a concentration of one $\mu$g per ml of medium. Substitutions in the *para* position of the benzene nucleus or in the 4-position of the thiosemicarbazone portion of the molecule reduce virostatic activity. 2. When fed in the diet, benzaldehyde thiosemicarbazone produces a marked degree of protection of mice inoculated intracerebrally with vaccinia virus. Derivatives with substitutions in the *para* position of the benzene nucleus or in the 4-position of the thiosemicarbazone portion of the molecule produce little or no protection against vaccinia infection in mice. 3. The thiosemicarbazones do not appear to inactivate vaccinia virus as the result of prolonged contact *in vitro*.

7. Donovick, R., Pansy, F., Stryker, G., and Bernstein, J., *J. Bact.*, 1950, v59, 667.

8. Hoggarth, E., Martin, A. R., Storey, N. E., and Young, E. H. P., *Brit. J. Pharmacol.*, 1949, v4, 248.

9. Hamre, D., Bernstein, J., and Donovick, R., *J. Bact.*, 1950, v59, 675.

10. Domagk, G., *Am. Rev. Tuber.*, 1950, v61, 8.

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