duce survival of 90 to 100% of burned mice challenged locally with pseudomonas strain 180(1). It was observed that this treatment brought about a pronounced increase in viable tail length, with 30 to 47% viable after 16 days and 35 to 50% after 30 days. Chloramphenicol, penicillin, and tetracyclin, which were without effect on survival when administered systemically, gave results similar to the untreated controls (Fig. 2); this absence of effect of chloramphenicol is in contrast with its activity when applied locally, as shown above. The results with systemic sulfadiazine and streptomycin are comparable to those obtained with effective local treatment. These findings indicate that sulfadiazine and streptomycin administered systemically not only protect the animals from generalized infection with pseudomonas 180, but also suppress the local infection in the tail. They also stress the importance in wound healing of pathogenic bacterial contamination.

The technics described here may prove useful for the study of various types of injury such as irradiation and chemical trauma, as well as the study of therapeutic agents on wound healing in the presence or absence of infection.

The length of the rodent tail makes it quite vulnerable to injury applied over its entire length, so that viable tail may afford a more sensitive index of tissue damage than other areas of body surface. A feature of the present study is the striking extent to which an injury, normally leading to the death of tissue, may be reversed by therapeutic measures.

Summary. A simple technic is described for measurement of wound healing following a standardized burn of the mouse tail. A procedure was also developed for the application of local therapy to the burned area. A marked influence on viable tail length was obtained by local protective measures, and by administration of local and systemic antibacterial therapy.

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Received May 5, 1966. P.S.E.B.M., 1966, v123.

Drug Interactions Between Disulfiram and α-Methyldopa and Related Agents in Reserpine-Pretreated Rats.* (31485)

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Tyramine owes its sympathomimetic effects to the release of norepinephrine from storage sites in postganglionic adrenergic nerves(1-3). The pressor response to tyramine and adrenergic nerve stimulation is reduced or abolished in animals pretreated with reserpine (1,4). The sympathetic nerve blockade by reserpine can be partially reversed by injection of dopa, dopamine, or norepinephrine (5,6). The α-methylated analogues of the catecholamines also reverse the nerve blockade by reserpine(6,7) and the tyramine blockade by reserpine(6,8).

The enzyme, dopamine-β-hydroxylase, catalyzes the conversion of dopamine to norepinephrine and other analogue of phenylethylamine to their corresponding β-hydroxylated derivatives (See review, 9). Disulfiram and other copper chelating agents are inhibitors of this enzyme *in vivo* as well as *in vitro*(10-12).

In the following experiment the effect that disulfiram and its active metabolic product, diethyldithiocarbamic acid(10), has on dopa, dopamine, α-methyldopamine and α-methyl-

* Supported by State of Washington Initiative 171 Funds for Research in Biology and Medicine.

TABLE I. Blood Pressure Increases from Tyramine.

Treatment	n	Tyramine response in mm Hg ± S.E. .5 hr	1.0 hr	1.5 hr
α-methyldopa after reserpine	5	53 ± 2	70 ± 3	76 ± 4
" after reserpine + disulfiram	3	32 ± 2‡	36 ± 4‡	37 ± 4‡
" after reserpine + DEDTC§	4	18 ± 8†	24 ±10†	30 ±12†
α-methyldopamine after reserpine	4	34 ±10	49 ±12	49 ±12
" after reserpine + disulfiram	3	35 ±10	25 ± 7	25 ± 8
" after reserpine + DEDTC	3	14 ± 6	16 ± 4*	15 ± 5*
Dopa after reserpine	4	30 ± 2	20 ± 2	18 ± 6
" after reserpine + disulfiram	5	24 ± 2*	24 ± 3	23 ± 4
" after reserpine + DEDTC	4	14 ± 3†	12 ± 4*	9 ± 4
Dopamine after reserpine	3	34 ±11	17 ± 2	12 ± 4
" after reserpine + disulfiram	3	22 ± 4	15 ± 1	12 ± 0
" after reserpine + DEDTC	3	6 ± 2*	5 ± 2*	7 ± 1
Norepinephrine after reserpine	4	38 ± 6	30 ± 5	22 ± 2
" after reserpine + disulfiram	4	25 ± 6	15 ± 7	15 ± 6
" after reserpine + DEDTC	5	22 ± 4*	17 ± 3*	8 ± 3†

Showing the increased blood pressure from tyramine in mm Hg ± standard error (S.E.) of the mean after treatment with α-methyldopa 75 mg/kg, α-methyldopamine 2 mg/kg, dopa 50 mg/kg, dopamine 1 mg/kg, and norepinephrine 0.025 γ/sec for 10 min.

* $P < .05$ with respect to reserpine treated group.
† $P < .01$ " " " " " " .
‡ $P < .001$ " " " " " " .
§ DEDTC = Diethyldithiocarbamic acid.

dopa to restore the tyramine response will be studied. It is proposed that an effective *in vivo* test for inhibitors of dopamine-β-hydroxylase is available using the α-methyldopa reversal of tyramine blockade by reserpine.

Methods. Sprague-Dawley rats, 225 to 300 g, are used in these experiments. The animals are anesthetized with pentobarbital, 30 mg/kg. Blood pressure from the left common carotid artery is recorded on a physiograph. Intravenous injections are made by cannulating the right jugular vein. Reserpine, 5 mg/kg, (Serpasil, Ciba) is injected on the day before and 2.5 mg/kg on the day of the experiment. Disulfiram is administered on the day before and on the day of the experiment, 400 mg/kg and 200 mg/kg respectively. Diethyldithiocarbamic acid is used in the same manner as disulfiram.

Tyramine, 0.5 mg/kg, is given to the reserpinized rats at ½ hour intervals 2 times before and 3 times after the administration of α-methyldopa or the other phenylethylamine analogues. Mean blood pressure is measured and reported.

The phenylethylamine analogues are given intravenously at the following dosages: α-methyldopa, 75 mg/kg, α-methyldopamine†, 2 mg/kg, dopa (dihydroxyphenylalanine), 50 mg/kg, and dopamine, 1 mg/kg. Norepinephrine is infused slowly for 10 minutes at a rate of 0.025 γ/sec.

Results. Table I shows that after the administration of α-methyldopa there is a 4-fold increase (Table II) in the pressor response to tyramine. Disulfiram and diethyldithiocarbamic acid greatly diminish the response to tyramine after α-methyldopa. α-methyldopamine also reverses the reserpine block of the pressor response to tyramine; however the reversal is not as great as in the case of α-methyldopa. Disulfiram does not have the blocking effect with α-methyldopamine at 0.5 hour that it has with α-methyldopa, but the 1 and 1.5 hour doses of tyramine give responses which are diminished but not statistically significant. α-methyldopamine itself gives an increase in blood pressure. Diethyldithiocarbamic acid appears to block the reversal of α-methyldopamine much more effectively than does disulfiram.

Dopa is not as effective in reversing the reserpine block of tyramine at this dose as the

† Kindly supplied by Dr. C. A. Stone, Merck Inst. for Therapeutic Research.

TABLE II. Blood Pressure from Phenylamines.

Phenylethylamine analogue	Dose	Reserpine	Reserpine + disulfiram	Reserpine + DEDTC‡
α-methyldopa	75 mg/kg	13 ± 5 (5)	20 ± 4 (3)	6 ± 2 (4)
α-methyldopamine	2 "	88 ± 5 (4)	82 ± 1 (3)	84 ± 7 (3)
Dopa	50 "	51 ± 7 (4)	56 ± 6 (5)	33 ± 7 (4)
Dopamine	1 "	70 ±10 (3)	83 ±10 (3)	72 ±11 (3)
Norepinephrine	10.5 γ	68 ± 6 (4)	90 ± 3* (4)	80 ± 7 (5)
Tyramine	.5 mg/kg	19 ± 1 (19)	15 ± 2 (18)	8 ± 1†(19)

Gives the increased blood pressure in mm Hg ± standard error of the mean. Drugs are administered as described in text.

* $P < .05$ with respect to reserpine treated group.

† $P < .001$ " " " " " " .

‡ DEDTC = Diethyldithiocarbamic acid.

α-methyl analogue of dopa. The dopa tends to be toxic in comparison to α-methyldopa as noted in a high mortality of rats within a short period after the injection. However, there is more than a 2-fold increase in the pressor response to tyramine after dopa. Disulfiram appears relatively ineffective in blocking this increased response, but diethyldithiocarbamic acid does cause some blockade of the dopa reversal. Dopamine at this dose weakly reverses the reserpine block of the tyramine pressor response. Disulfiram does cause some tendency toward inhibition of the weak reversal, but diethyldithiocarbamic acid significantly inhibits the reversal.

Dopamine like dopa and α-methyldopamine give by themselves a significant increase in blood pressure and these responses are not inhibited by either disulfiram or diethyldithiocarbamic acid (Table II). The norepinephrine response is seen to be potentiated in the presence of disulfiram. The pressor response to tyramine after diethyldithiocarbamic acid is greatly diminished.

Norepinephrine is administered to see the effect that disulfiram and diethyldithiocarbamic acid might have on its ability to reverse the reserpine blockade of tyramine. There is a small effect by disulfiram and a significant effect by diethyldithiocarbamic acid.

Studies not in the Tables demonstrated that neither disulfiram nor diethyldithiocarbamic acid inhibits tyramine response in control animals not pretreated with reserpine.

Discussion. In these experiments α-methyldopa is most effective in causing a reversal of the reserpine blockade of tyramine. Because it has no significant effect itself on these anesthetized animals, it is used in comparatively high doses. Disulfiram and diethyldithiocarbamic acid are able to inhibit the reversal by about 50%, the diethyldithiocarbamic acid being slightly more effective than disulfiram.

It is peculiar to α-methyldopamine that the reversal of the resserpine block of tyramine is not significantly blocked by disulfiram; however, diethyldithiocarbamic acid is effective in blocking. α-methyldopamine gives a pressor response itself, and it is possible that α-methyldopamine is taken up and released by the first dose of tyramine. This would explain the large response when tyramine is used in the disulfiram pretreated rats. However, this does not occur in the case of diethyldithiocarbamic acid. Both dopa and dopamine are inhibited by diethyldithiocarbamic acid.

Most surprising are the results with norepinephrine infusions and treatment with disulfiram and diethyldithiocarbamic acid. It is evident that norepinephrine does effectively reverse the reserpine blockade of the pressor response to tyramine. Musacchio *et al*(12) have shown tha disulfiram does not interfere with H^3-norepinephrine in the heart. From these experiments it can be assumed that there is a partial block of the response to norepinephrine by disulfiram and a significant block by diethyldithiocarbamic acid. It is also notable that although a partial block of the reversal is evident by these agents, there is in the same rats an increased response to the infused norepinephrine. This potentiated response seems to indicate a sensitization of the vasculature to norepinephrine or a decreased

uptake and thereby prolonged action. There is a tendency, not statistically significant, for α-methyldopa, dopa and dopamine to be also potentiated by disulfiram. It is evident that the actions of disulfiram and diethyldithiocarbamic acid are much more complex than just an inhibition of dopamine-β-hydroxylase. In the case of these two inhibitors, especially diethyldithiocarbamic acid, their block of the norepinephrine reversal effect of the reserpine block of tyramine makes this test for dopamine-β-hydroxylase questionable.

Diethyldithiocarbamic acid is more effective than disulfiram in inhibiting α-methyldopa, α-methyldopamine, dopa, and dopamine reversal of the reserpine block of the pressor response of tyramine.

It is possible that diethyldithiocarbamic acid is also preventing decarboxylation as the 2 carboxylated agents, α-methyldopa and dopa, are inhibited more by diethyldithiocarbamic acid than by disulfiram.

Summary. In these experiments rats are reserpinized, and it is demonstrated that α-methyldopa reverses the tyramine pressor response blockade by reserpine. Diethyldithiocarbamic acid will inhibit the reversal process by α-methyldopa· α-methyldopamine, dopamine, and dopa. Disulfiram inhibits the reversal process of α-methyldopa and dopa. Disulfiram or diethyldithiocarbamic acid alone will not inhibit the tyramine response. Diethyldithiocarbamic acid is most potent of the two agents used in inhibiting the reversal process.

ADDENDUM: Musacchio, J. M., Bhagat, B., Jackson, C. J. and Kopin, I. J., J. Pharmacol., 1966 v152, 293, have used nearly the same technique to show inhibition by disulfiram of the restoration by dopamine and α-methyldopamine of the response to tyramine in reserpine-treated cats.

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Received May 31, 1966. P.S.E.B.M., 1966, v123.

In vitro Effects of Zinc on Insulin Activity in Adipose Tissue.* (31486)

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Since the isolation and crystallization of insulin by Scott in 1934(1), zinc has been associated with the insulin molecule. However, the role, if any, of zinc in insulin metabolism and whether it is required for the hormonal action of insulin has not yet been determined. Baker and Rutter(2) have commented that media containing more than 10^{-5} M zinc inhibited glucose uptake by isolated rat epididymal adipose tissue in the presence of insulin. Recently, in this laboratory we have been impressed both in *in vivo* and *in vitro* studies by the action of zinc in

* Supported by USPHS Grant AM03056
Fund for Research and Teaching, Dept. of Nutrition, Harvard School of Public Health.