the infectious Brunhilde particles were stable. If at least the major part of the phenol-stable ribonuclease-labile poliovirus infectivity is *produced* by phenol treatment, as it apparently is in the case of Mengo encephalitis virus(3), then the finding that this infectivity is present in its maximal titer (maximal for assay system employed) within not more than 0.5 minute, while the original ribonuclease-stable virus population disappears within not more than this same period of time, suggests that both this disappearance and the appearance of the phenol-stable ribonuclease-labile infectivity may be the result of a single fast reaction.

*Summary.* The influence of phenol concentration, time, and pH on phenolic inactivation of poliovirus at 0° was studied. A minimal phenol concentration of about 0.5 M was required for removal of all the original ribonuclease-stable poliovirus infectivity. This minimal requirement was influenced slightly by pH; it was lower at pH 4.0 than at pH 6.2 and lower at pH 6.2 than pH 7.4. Amount of inactivation was independent of time of exposure to phenol over range of 0.5 to 240 minutes. Poliovirus populations studied were heterogeneous with respect to phenol-stability, some particles requiring higher phenol concentrations than others for inactivation. Under conditions giving phenolic inactivation of all ribonuclease-stable infectivity, the ribonuclease-labile infectivity was present 0.5 minute after start of reaction and its titer remained essentially constant for at least 4 hours.

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## Pharmacological Studies with Nialamide, A New Antidepressant Agent. (25112)

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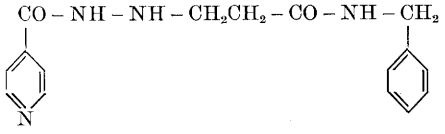
The introduction of monoamine oxidase (MAO) inhibitors as antidepressants has opened up a new concept in chemotherapy of mental disorder. As a result of favorable modification of psychic state of patients during its clinical trial as a tuberculostat, iproniazid was investigated intensively in depressed patients(1) and had marked antidepressant properties. Since side effects such as postural hypotension(2) and hepatotoxicity(3) were encountered, it was necessary to search for agents which lacked these attributes but retain the therapeutic value. From a large series of carboxamido alkyl hydrazines(4) investigated for this purpose, nialamide,*



was chosen for further studies because its out-

* Generic name of Niamid, Chas. Pfizer & Co.

standing *in vivo* and *in vitro* MAO inhibitor potency and lack of side effects(5). We present some important pharmacological studies with nialamide, with particular emphasis on its antidepressant activity.

*Materials and methods.* 2,900 male Swiss Webster mice, 40 rabbits, 50 cats and 40 dogs were used. Although a causal relationship between MAO inhibition and antidepressant action has not been established, there is close correlation of these in some of the agents currently used to treat depressed psychotic patients. We therefore chose the following measures for assessing activity of nialamide as an antidepressant: antagonism of reserpine depression, potentiation of 5-hydroxytryptophan (5-HTP) and MAO inhibition *in vivo*. *Reserpine antagonism.* Male albino mice weighing 17 to 25 g were pre-treated with MAO inhibitor either orally or intraperitoneally followed 18 hours later by intraperitoneal injection of reserpine, 5 mg/kg. Three and 4 hours after reserpine injection, the amount of ptosis and decrease in spontaneous motor activity of animals was scored. Degree of reserpine antagonism was calculated from the ratio of difference between score for reserpine control and that for the pre-treated mice, compared to the former, expressed as percent. A similar procedure was followed with cats and dogs. In these animals, the MAO inhibitor was given orally, and dose of reserpine, 1 mg/kg, given intravenously. *Pyretogenic response to 5-HTP.* An alternative means of measuring MAO inhibitory effect was suggested by Horita and Gogerty(6) using potentiation by MAO inhibition of the pyretogenic response of rabbits to 5-HTP. Rabbits were pre-treated by subcutaneous injection of MAO inhibitor 18 hours before intravenous injection of 5-HTP, 25 mg/kg. Rectal temperature was taken at 30 minute intervals both before and on day of test. Nialamide had a negligible effect on body temperature. Only the highest dose, 32 mg/kg, caused a significant increase (0.41°C, $P = 0.04$) over the previous day. Following injection of 5-HTP, peak temperature occurred in about 3 hours. *In vitro* analysis of dog brain stem for MAO activity and for serotonin and norepinephrine content was done by modification of the Bogdanski method(7). Monoamine oxidase activity in this tissue was assayed using serotonin as substrate. Tissues were taken from animals which received daily doses 5 days or 6 months. The time course of action of nialamide was followed quantitatively by determining degree of protection by nialamide against reserpine facilitation of Metrazol for 11 days. Albino mice were infused intravenously with 0.5% solution of Metrazol at 0.05 ml/10 sec until onset of tonic convulsions(8). Reserpine, 5 mg/kg injected intraperitoneally 3 hours prior to Metrazol infusion, lowers the threshold to Metrazol-induced convulsions to approximately 37% of control value. The ability of MAO inhibitors to potentiate hexobarbital sleeping time was determined in albino mice. Animals were injected with the inhibitors 18 hours before, 1 hour before, or simultaneously with intraperitoneal injection of hexobarbital, 100 mg/kg, and duration of sleeping time determined. Loss and recovery of righting reflex was taken as onset and end point of sleep.

*Results. Symptomatology.* Nialamide alone caused few overt symptoms in mice. Normal animals treated orally or parenterally in dose range to 100 mg/kg showed remarkably little effect attributable to the drug. A dose of 100 mg/kg caused slight decrease in spontaneous motor activity for about 60 minutes followed by mild increase lasting about 24 hours. At 200 mg/kg the effect was somewhat more noticeable but not marked. Intraperitoneal and oral $LD_{50}$ with 95% confidence limits in mice were 742 (584-942) and 1,000 (741-1358) mg/kg, respectively(9,10). At toxic doses irritability, tremors, and salivation were observed, all of which are manifestations of sympathetic stimulation.

*Reserpine antagonism.* To compare the antidepressive potency of nialamide with that of iproniazid and other agents, reserpine antagonism was used. Previous administration of monoamine oxidase inhibitor postponed the onset and in some cases altered the character of depression resulting from reserpine. The intensity of this action was proportional to dose. Nialamide had about 2 to 3 times the

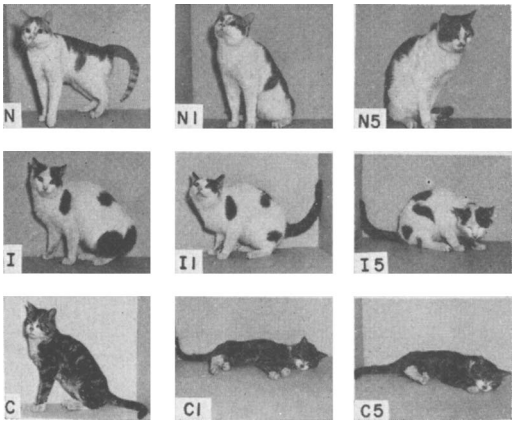



FIG. 1. Reserpine effect in cats following MAO inhibition. N, 18 hr after nialamide, 5 mg/kg, P.O.; I, 18 hr after iproniazid, 40 mg/kg, P.O.; C, control, no treatment; N1, I1, C1: 1.5 hr after reserpine, 1 mg/kg, I.V. N5, I5, C5: 5 hr after reserpine, 1 mg/kg, I.V.

potency of iproniazid in dogs and about 10 times in mice and cats (Fig. 1).

*Potentiation of pyretogenic action of 5-HTP.* Horita(11) used potentiation of the pyretic response to 5-HTP to compare potencies of MAO inhibitors. This procedure was used to compare potencies of iproniazid and nialamide (Fig. 2). By this test nialamide was about 2.5 times more potent than iproniazid.

*In vitro* analysis was done for serotonin and norepinephrine content as well as monoamine oxidase activity of brain stem tissue from dogs given nialamide, iproniazid, or β-phenylisopropylhydrazine (PIH). Differences in potency observed in intact animals correlated with degrees of inhibition of the enzyme and elevation of serotonin content (Fig. 3, Table I). From these results a difference in enzymatic action between nialamide and the others became evident. Whereas they all caused elevation of serotonin, nialamide also increased norepinephrine content. Brain stem tissue of dogs examined after 6 months continuous dosage *i.e.*, 1 to 30 mg/kg/day showed the same changes in amine content and enzyme activity indicating very little cumulative effect with prolonged treatment (Table I).

TABLE I. Serotonin and Norepinephrine Content of Dog Brain Stem following Nialamide.

| Inhibitor | Length of treatment | Daily dose, mg/kg P.O. | Brain stem amine content 5 HT, μ/g | NE, μ/g |
|---|---|---|---|---|
| Controls | 5 days | | .52 | .32 |
| Nialamide | " | 50 | 1.0 | .61 |
| PIH | " | 10 | 2.8 | .26 |
| Iproniazid | " | 50 | 1.0 | .35 |
| Controls | 6 mo | | .55 | .21 |
| Nialamide | " | 1 | .57 | .28 |
| " | " | 3 | .57 | .25 |
| " | " | 10 | 1.25 | .50 |
| " | " | 30 | 1.54 | .52 |

*Duration of action.* The degree of reserpine antagonism due to a 10 mg/kg dose of nialamide reached a maximum in about 1-2 hours, then fell slowly over a period of days, a significant portion of initial activity still present

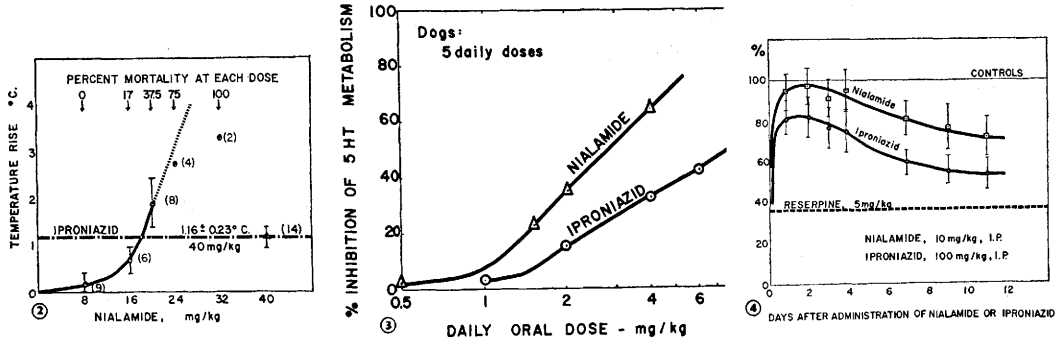



FIG. 2. Potentiation of 5-HTP pyretogenesis; peak temp. rise ± S.E., about 3 hr after inj. of 5-HTP, 25 mg/kg, I.V.; nialamide, S.C., 18 hr before 5-HTP; No. of rabbits/dose in parentheses.

FIG. 3. Brain MAO inhibition.

FIG. 4. Duration of action, protection against reserpine facilitation of Metrazol-induced convulsions, vertical axis: % of tonic convulsant volume of Metrazol for controls; Metrazol, I.V., 0.5% solution, 0.05 ml/10 sec.; reserpine, I.P., 5 mg/kg, 3 hr before Metrazol infusion; 12 mice/treatment; vertical bars indicate stand. error.

TABLE II. Effect of Nialamide on Hexobarbital Anesthesia.

| Treatment* | Inj. time† | Duration‡ LRR, min. | Ratio T/C§ | △ means, min.‖ | $^{s}$△, min. | t | d.f. | P |
|---|---|---|---|---|---|---|---|---|
| Nialamide + hexo. | Simult. | 70.7 | 2.86 | 46.0 | 3.6 | 12.7 | 22 | $<<0.001$ |
| Hexobarb. control | | 24.7 | | | | | | |
| Nialamide + hexo. | 1 hr | 44.6 | 3.0 | 29.9 | 4.85 | 6.2 | 18 | " |
| Hexobarb. control | | 14.7 | | | | | | |
| Nialamide + hexo. | 16 " | 31.4 | .8 | −10.1 | 4.86 | 2.1 | 18 | 0.06 |
| Hexobarb. control | | 41.5 | | | | | | |
| Iproniazid + hexo. | Simult. | 132.6 | 6.9 | 113.5 | 7.3 | 15.5 | 22 | $<<0.001$ |
| Hexobarb. control | | 19.1 | | | | | | |
| Iproniazid + hexo. | 1 hr | 95.4 | 7.6 | 82.8 | 5.1 | 16.3 | 18 | " |
| Hexobarb. control | | 12.6 | | | | | | |
| Iproniazid + hexo. | 18 " | 15.6 | .7 | −6.1 | 1 | 6.1 | 18 | " |
| Hexobarb. control | | 21.7 | | | | | | |
| Nialamide¶ + hexo. | Simult. | 121.7 | 4.8 | 96.6 | 10.2 | 9.5 | 22 | " |
| Hexobarb. control | | 25.1 | | | | | | |

* Nialamide, 10 mg/kg, IP; iproniazid, 100 mg/kg, IP; hexobarbital, 100 mg/kg, IP. † Time between inj. of inhibitor and hexobarbital. ‡ Mean duration of loss of righting reflex. § Ratio of mean duration of treated to that of control. ‖ Difference between mean duration of treated and control. ¶ Nialamide, 100 mg/kg, IP.

after 12 days (Fig. 4).

*Hexobarbital potentiation.* Potentiation of hexobarbital sleeping time by iproniazid has been reported by Fouts and Brodie(12). Inhibition of oxidation of the side chain is the mechanism proposed. Plaa, Evans, and Hines(13) utilized potentiation of pentobarbital by a series of halogenated hydrocarbons as indication of degree of interference with liver function and found good correlation with histologic signs of toxicity. With this in mind, nialamide and iproniazid were compared for hexobarbital potentiation at doses which produced equivalent degrees of reserpine antagonism, 10 and 100 mg/kg respectively. Whereas iproniazid prolonged sleeping time 6.9 times, nialamide potentiation was only 2.9 times that of controls (Table II). That this effect was unrelated to monoamine oxidase inhibition was suggested by difference in time of reserpine antagonism and hexobarbital potentiation, the latter reaching a peak about 1 hour after administration and essentially absent 18 hours later (in preparation, R. P. Rowe, *et al.*)

*Discussion.* The antidepressant potency of nialamide has been demonstrated to be greater than that of iproniazid as judged by reserpine antagonism, 5-HTP potentiation, and *in vitro* monoamine oxidase inhibition. Although difference in activity between nialamide and iproniazid varied from species to species, the former was always more active. Similar potency of parenteral and oral dosage indicates good absorption of nialamide.

The most important aspect of this study concerns the correlation of animal tests with clinical results. No proof is available as yet implicating a causal connection between monoamine oxidase inhibition and antidepressant activity in the human. However, it has been observed that in humans monoamine oxidase inhibition may be demonstrated with nialamide(14) at approximately the same dose that gives a therapeutic effect in depressed patients (15).

Iproniazid and other monoamine oxidase inhibitors raised the serotonin content of dog brain, while norepinephrine levels were not significantly altered in this species. However, nialamide also caused an elevation of norepinephrine. Whether this factor is in any way related to lack of hypotensive activity of nialamide reported in humans(14,15) has not been answered.

Other differences between nialamide and iproniazid are demonstrable. Thus, the prolonged hexobarbital sleeping time after iproniazid contrasts with mild potentiation of this phenomenon with nialamide. Prolongation of hexobarbital sleeping time has been utilized as an indication of interference with liver func-

tion and consequent toxicity. This is in accord with chronic toxicity experiments in dogs (10) where nialamide had no functional, gross anatomical, or histological effects on the liver.

*Summary.* Nialamide was a potent monoamine oxidase inhibitor by both *in vitro* and *in vivo* test. Its potency relative to that of iproniazid varies from 3:1 to 12:1 depending on test and species used. Nialamide was also shown to be qualitatively different from other MAO inhibitors in some of its pharmacological actions, factors which may explain absence of both liver toxicity in dog and postural hypotension in man.

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## Experimental Detection of Left-to-Right Circulatory Shunts with Injections of Krypton$^{85}$. (25113)

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Improved methods for detection of circulatory shunts have evolved in recent years. Dye-dilution curves and inhaled foreign gas technics(1,2,3) have both proved superior to oximetry in detection and localization of left-to-right shunts. Our experience has demonstrated the usefulness of solutions of radioactive krypton gas ($Kr^{85}$) in detection and localization of right-to-left shunts. By continuously monitoring the expired air with a Geiger-Müller tube, appearance time and concentrations of krypton$^{85}$ in expired gases can be determined. When $Kr^{85}$ is injected into the right side of heart or pulmonary artery, it appears immediately and in high concentrations but when injected into the left side of heart the appearance time is long and initial concentration is low. This observation suggested that the early appearance of $Kr^{85}$ in expired air after its injection into the left side of heart would be indicative of a left-to-right shunt.

*Materials and methods.* Left-to-right shunts were constructed in 8 mongrel dogs by anastomosis of the left subclavian artery to the left pulmonary artery. Anesthesia was induced with sodium pentobarbital, a cuffed-endotracheal tube was inserted and positive pressure respiration was maintained at a rate of 20/min by a demand respirator. Seven dogs were studied in acute experiments and one was catheterized 2 weeks after operation. A #6 Cournand catheter was inserted *via* femoral artery to the root of the aorta. Injections of krypton$^{85}$ in saline solution were made at the aortic valve with the shunt open and after it was occluded. Patency of anastomosis was