citement is not seen nor can anesthesia and medullary depression be induced by increasing the dose. Unlike the tranquilizers, this thiazolothiazole derivative does not alter autonomic function or exhibit evidences of central stimulation.

EXPERIMENTAL

General Procedures.—The molar ratio of reactants and other reaction data appear in Table I. *Procedure A.*—Following the published procedure (2), a mixture of dithio-oxamide and the aldehyde was heated under a stream of nitrogen in a wax bath. The reaction mixture was cooled and treated with ethanol. The product was filtered and recrystallized.

Procedure B.—Procedure A was modified by refluxing the reactants in dry pyridine for 60 hours. After cooling, the product was filtered and recrystallized.

Dihydrazide of Thiazolo[5,4-d]thiazole Dicarboxylic Acid.—A mixture of 0.5 Gm. (2 mmoles) of bis-(carbomethoxy)-thiazolothiazole, prepared from the corresponding acid (2) and diazomethane, 325 mg. (6 mmoles) of hydrazine hydrate (85% aqueous solution) and 125 ml. of ethanol was refluxed for 6 to 7 hours. The reaction mixture was concentrated by distillation under reduced pressure, filtered, and the product washed with ethanol. The yield was practically quantitative. A suitable recrystallization procedure was not achieved. The crude washed product, m.p. > 400°, gave the following analytical results.

Anal.—Calcd. for $C_6H_6N_6O_2S_2$: N, 32.56; S, 24.84. Found: N, 31.20; S, 24.78.

Diisopropylidenehydrazide of Thiazolo[5,4-d]thiazole Dicarboxylic Acid.—Five drops of glacial acetic acid was added to a suspension of 0.5 Gm. (2 mmoles) of the dihydrazide of thiazolothiazole dicarboxylic acid in 100 ml. of reagent acetone. After refluxing for 1 hour, the reaction mixture was concentrated under reduced pressure and filtered. The yield was practically quantitative. After recrystallization from acetone-ethanol, the product melted at 296–298°.

Anal.—Calcd. for $C_{12}H_{14}N_6O_2S_2$: N, 24.84; S, 18.95. Found: N, 25.28; S, 18.60.

Diamide of Thiazolo[5,4-d]thiazole Dicarboxylic Acid.—A fine suspension of bis-(carbomethoxy)-thiazolothiazole was prepared by dissolving 0.2 Gm. (0.78 mmole) of the ester in 5 ml. of boiling dioxane, followed by rapid cooling in an ice bath. Five milliliters of ammonium hydroxide (27%) was added and the mixture allowed to stand overnight at room temperature. After concentrating the mixture to one-half volume under reduced pressure, the white precipitate was filtered and washed with water and ethanol. The yield was practically quantitative. A suitable recrystallization procedure was not achieved. The crude, washed product did not melt when heated to 400°.

Anal.—Calcd. for $C_6H_4N_4O_2S_2$: N, 24.55; S, 28.00. Found: N, 24.30; S, 27.86.

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Pantothenyl Alcohol Effect on Delta-1-Cortisol-Induced Gastric Ulcers

By THOMAS A. LYNCH, WILBUR L. HIGHLEY, and ALBERT G. WORTON

The development of a standardized technique in assessing the ulcerogenic side effects of the corticosteroid drugs has provided an effective means of evaluating antiulcer agents. Approximately 90% incidence of gastric ulcers can be produced in fasting female rats by daily subcutaneous administration of 40 mg./Kg. delta-1-cortisol (D1C) for 4 days. Pantothenyl alcohol, extensively used in treating postoperative intestinal atony, was evaluated for antiulcer activity in such treated animals by two methods: (*a*) oral feeding during the 4 days induction period to determine effect on development on ulcers and (*b*) oral feeding after induction of ulcers to determine the effects on spontaneous healing. Pantothenyl alcohol did not reduce the degree of ulceration during the induction period, but markedly accelerated spontaneous ulcer healing.

THE IMPORTANCE of pantothenic acid to gastrointestinal integrity has been demonstrated by studies involving pantothenic acid deprivation and the pituitary axis. In 1943, Bly, *et al.* (1), demonstrated a 50% reduction of intestinal motility in pantothenic acid-deficient dogs. About the same time, Jurgens and Pfaltz (2) observed atony and distention of the gastrointestinal tract of pantothenic acid-deficient rats. These observations helped establish the basis for the use of pantothenic acid in the treatment of postoperative intestinal atony and gaseous intestinal distention (3–6). The discovery by Lipmann, *et al.* (7), that pantothenic acid formed an integral part of coenzyme A, and the pantothenic deficiency studies in humans by Bean,

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et al. (8), were important in establishing the link between pantothenic acid and intestinal formation of acetylcholine.

The relationship of pantothenic acid in resistance to stress and adrenocortical function has been presented by numerous workers (9–14). These studies implicate pantothenic acid as a protective factor against certain nonspecific stress effects.

Evidence for the correlation of the ulcerogenic effects of stress and adrenocortical activity has been demonstrated (15–21). However, Zucker (22) was the first to implicate pantothenic acid as a factor in ulcerogenesis. He demonstrated the formation of duodenal ulcers in rats on a pantothenic acid-deficient diet, which were healed by pantothenic acid administration. Evidence was presented in his study to show that the intact adrenal cortex and pituitary gland were necessary for development of these ulcers. An increase in sensitivity and vulnerability of the duodenal mucosa, as a target area, to the effects of adrenocortical hormone was suggested as a mode of ulcerogenesis.

Since there appeared to be good evidence that pantothenic acid deficiency was implicated in the etiology of experimental ulcers, it was decided to study the effect of pantothenyl alcohol in the prevention and treatment of such experimental ulcers in rats without a pantothenic acid depletion.

EXPERIMENTAL

The method of producing gastric ulcers was that of Robert and Nezamis (17), which consisted of administering daily subcutaneous doses of D1C for 4 days to fasted female rats. The animals were sacrificed with chloroform, the stomach removed, cut along the lesser curvature, and the mucosa grossly examined for incidence and severity of ulcers. A composite ulcer index was devised which measured the number, size, and per cent incidence of the ulcers.

Preventive Effects of Pantothenyl Alcohol in D1C-Induced Gastric Ulcers in Rats.—The method of producing ulcers in this study was essentially the same as described by the original workers (17). In the first part of this experiment, pantothenyl alcohol was administered during the ulcer induction period. Thirty female Wistar rats, weighing 167–236 Gm., were used. The animals were divided into three groups using five rats per cage. D1C suspended in a Tween base[1] was administered subcutaneously to two groups of 10 animals each, using a dosage of 1 ml. (40 mg.)/Kg. daily. Ten control rats were given an equivalent dosage of the vehicle by the subcutaneous route. Of the D1C-treated animals, 10 were given 1 mg./ml. of pantothenyl alcohol in their drinking water. All animals were sacrificed with chloroform after 4 days of steroid treatment and fasting, the stomachs removed, and the ulcer index determined. In addition, the adrenal glands were removed and weighed.

Pantothenyl Alcohol Treatment of D1C-Induced Gastric Ulcers in Rats.—Under similar conditions, the effect of pantothenyl alcohol was determined on the healing rate of gastric ulcers after the 4 days ulcer induction period. Thirty female Wistar rats, weighing 160–195 Gm., were used. All animals were given D1C subcutaneously under the same conditions as above. Following 4 days of ulcerogenic treatment, 25 mg./Kg. of pantothenyl alcohol was administered subcutaneously to 15 rats, which thereafter were given 1 mg./ml., of pantothenyl alcohol in their drinking water. Control animals were given an equivalent volume of normal saline subcutaneously. All animals were on Purina laboratory chow diet for the first day, followed by powdered whole milk[2] the remainder of the time. Animals were then sacrificed with chloroform at daily intervals for 3 days to determine the ulcer indexes. Thymus and adrenal gland weights were recorded throughout the experiment. Hemoglobin, erythrocyte, and leucocyte levels were determined on the third day of treatment.

An additional 52 rats (151–191 Gm.) were studied as above to confirm the 3 days pantothenyl alcohol treatment results and to extend the observations over a longer period. The procedure was the same as before, except that Purina laboratory chow was resumed as the diet on the ninth day, and pantothenyl alcohol was withdrawn from the rats on the thirteenth day of the 16 days treatment period. Hematologic tests were not made.

RESULTS

Preventive Effects of Pantothenyl Alcohol in D1C-Induced Gastric Ulcers in Rats.—When pantothenyl alcohol was given during the D1C gastric ulcer induction period, no improvement in the ulcer index was observed. Fasted rats, treated with D1C for 4 days, showed an ulcer index of 16.7, compared to 18.4 for rats on D1C and pantothenyl alcohol (Table I). The ulcer index, as determined by the method of Robert and Nezamis (17), is broken down for the individual rat to demonstrate the severity and incidence. The incidence of ulcers was 95% for the 20 D1C treated rats. These ulcers were found in the pyloric part of the stomach only. An ulcer index of 9.05 was also calculated for the control animals receiving the vehicle alone. Here the ulcers were found in the forestomach only. The results in Table I agree very favorably with those of Robert and Nezamis (17).

As shown in Table II, D1C reduced the adrenal gland weight by about 15%, but pantothenyl alcohol did not significantly influence this change. The mean body weight losses of the control, D1C and D1C-pantothenyl alcohol groups, during the 4 days fast period, were 25.4, 32.3, and 35.5%, respectively. The mean daily water consumption was about 100 ml./Kg. in all groups.

Pantothenyl Alcohol Treatment of D1C-Induced Gastric Ulcers in Rats.—Failure of pantothenyl alcohol administration to affect the development of D1C-induced gastric ulcers, prompted the in-

[1] Tween base, 5 mg. methylcellulose, 4 mg. Tween 80, 9 mg. sodium chloride, and 9 mg. of benzyl alcohol per 1 ml. of aqueous solution.

[2] M & R Dietetic Laboratories Inc., Columbus, Ohio.

TABLE I.—EFFECT OF PANTOTHENYL ALCOHOL ON D1C-INDUCED GASTRIC ULCERS IN FEMALE WISTAR RATS, 167–236 Gm.

Rat No.	Control: No. of Ulcers	Control: Severity	D1C[a]: No. of Ulcers	D1C[a]: Severity	D1C[a] and Pantothenyl Alcohol[b]: No. of Ulcers	D1C[a] and Pantothenyl Alcohol[b]: Severity
1	0	...	8	17	5	14
2	4	11	6	11	6	10
3	3	6	7	19	1	2
4	0	...	0	...	1	3
5	0	...	9	19	1	2
6	4	11	3	6	8	14
7	0	...	8	20	11	17
8	2	5	4	6	15	35
9	1	3	6	21	5	13
10	0	...	3	6	7	33
Ulcer index	9.05[c]		16.7[d]		18.4[d]	

[a] D1C suspension, 1 ml. (40 mg.)/Kg. subcutaneously per day for 4 days. [b] Pantothenyl alcohol, 1 mg./ml. in drinking water, equivalent to approximately 100 mg./Kg. [c] Ulcers found in forestomach only. [d] Ulcers found in pyloric part of stomach only.

TABLE II.—ADRENAL WEIGHTS OF FASTED FEMALE WISTAR RATS SUBJECTED FOR 4 DAYS TO PANTOTHENYL ALCOHOL AND THE STEROID ACTIONS OF D1C

Treatment	Mean Weight,[a] mg./100 Gm. ± S.E.
Control	45.1 ± 3.32
D1C[b]	38.3 ± 1.72
D1C[b] and pantothenyl alcohol[c]	37.5 ± 2.49

[a] Calculated from 10 rats per group. [b] D1C suspension 1 ml. (40 mg.)/Kg. subcutaneously per day for 4 days. [c] Pantothenyl alcohol, 1 mg./ml. in drinking water, equivalent to approximately 100 mg./Kg. per day for 4 days.

TABLE III.—EFFECT OF PANTOTHENYL ALCOHOL FOLLOWING THE DEVELOPMENT OF GASTRIC ULCERS BY D1C[a] IN FEMALE WISTAR RATS, 160–195 Gm.

Days of Treatment	Control: No. of Rats	Control: Ulcer Index	Pantothenyl Alcohol[b]: No. of Rats	Pantothenyl Alcohol[b]: Ulcer Index
0	2	20.1	...	...
1	2	20.3	3	20.7
2	7	17.0	6	11.5
3	5	17.4	5	9.4

[a] D1C suspension, 1 ml. (40 mg.)/Kg. subcutaneously per day for 4 days in fasted rats. [b] Pantothenyl alcohol, 25 mg./Kg. subcutaneously, given immediately after the 4 days ulcer induction period; thereafter, 1 mg./ml., equivalent to about 300 mg./Kg. daily, given in the drinking water.

TABLE IV.—EFFECT OF PANTOTHENYL ALCOHOL FOLLOWING THE DEVELOPMENT OF GASTRIC ULCERS BY D1C[a] IN FEMALE WISTAR RATS, 151–191 Gm.

Days of Treatment	Control: No. of Rats	Control: Ulcer Index	Pantothenyl Alcohol[b]: No. of Rats	Pantothenyl Alcohol[b]: Ulcer Index
0	2	26.8	...	...
1	2	28.2	3	22.5
2	7	24.0	6	20.8
3	5	24.3	5	18.1
5	5	20.5	5	13.9
9	3	17.2	3	12.1
16	3	8.1[c]	3	0.0

[a] D1C suspension, 1 ml. (40 mg.)/Kg. subcutaneously per day for 4 days in fasted rats. [b] Pantothenyl alcohol, 25 mg./Kg. subcutaneously, given immediately after the 4 days ulcer induction period; thereafter, 1 mg./ml., equivalent to about 300 mg./Kg. daily, given in the drinking water. [c] One rat with perforated ulcer.

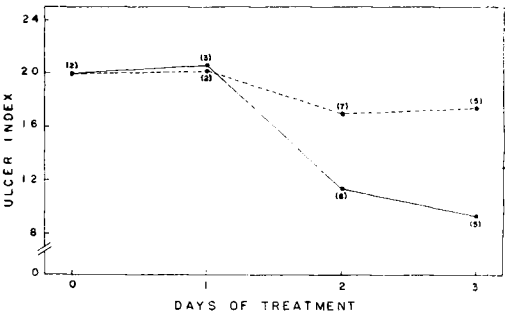

Fig. 1.—Effect of pantothenyl alcohol following the development of gastric ulcers in female Wistar rats. Ulcers induced by 40 mg./Kg. of D1C subcutaneously per day for 4 days in 30 fasted rats. Dotted line represents control animals. Unbroken line represents animals given 25 mg./Kg. of pantothenyl alcohol subcutaneously, immediately after the 4 days ulcer induction period, followed thereafter by 1 mg./ml. in the drinking water (equivalent to about 300 mg./Kg. daily). Number of animals in parentheses.

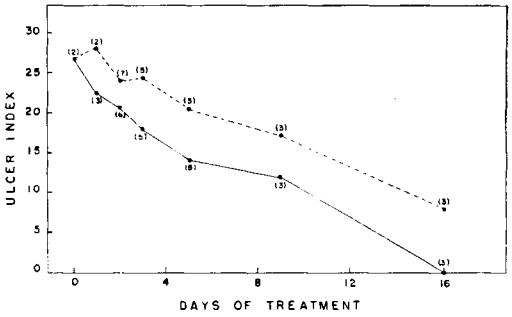

Fig. 2.—Effect of pantothenyl alcohol following the development of gastric ulcers in female Wistar rats. Ulcers induced by 40 mg./Kg. of D1C subcutaneously per day for 4 days in 52 fasted rats. Dotted line represents control animals. Unbroken line represents animals given 25 mg./Kg. of pantothenyl alcohol subcutaneously, immediately after the 4 days ulcer induction period, followed thereafter by 1 mg./ml. in the drinking water (equivalent to about 300 mg./Kg. daily). Number of animals in parentheses.

vestigation of pantothenyl alcohol effects on the spontaneous healing of ulcers. When pantothenyl alcohol was given on a treatment regimen to rats with D1C-induced gastric ulcers, definite declines below the control ulcer indexes were observed. Table III and Fig. 1 show a decrease of 32% in the ulcer index of pantothenyl alcohol-treated rats after 2 days of treatment and a 46% decrease after 3 days.

Hemoglobin, erythrocyte, and leukocyte levels on the third day of treatment were essentially the same in control and treated groups. Determination of thymus and adrenal gland weights failed to reveal a definite pattern.

Water consumption during the 4 days D1C treatment period was approximately 50% greater than before, but increased about twofold in both groups when food and treatment were instituted. Body weight, reduced about 33%, remained at this level in both groups even though the animals were

receiving food during the treatment period. Unabsorbed D1C remained in the subcutaneous site in all of the animals after 3 days of treatment and may have accounted for the absence of weight gain.

The results of the additional 52 rats tabulated in Table IV and depicted in Fig. 2, show an initially higher ulcer index than before, but essentially the same reduction of ulcer index by pantothenyl alcohol during the first 3 days of treatment. The ulcer indexes of the pantothenyl alcohol-treated rats were consistently lower than the controls and averaged about 23% below controls during the first 9 days of treatment. On the sixteenth day, no ulcers were found in the pantothenyl alcohol group, while one perforated ulcer was found in the control group. Extensive multiple erythematous eruptions of the forestomach occurred in both treated and control groups on the fifth day of treatment only. These were more severe in the control rats. No significant changes in thymus and adrenal gland weight patterns were noted.

The relative water consumption and weight change pattern during the first 3 days of treatment was essentially the same as noted earlier. Water consumption continued at about the same rate until the end of the experiment. After the fifth day of treatment, control rats began to gain weight, concomitant with the disappearance of D1C deposits in the subcutaneous tissues; however, on the ninth day, pantothenyl alcohol-treated animals showed obvious signs of alopecia and became emaciated, even though their ulcer index was declining more rapidly. When pantothenyl alcohol was withdrawn from the drinking water on the thirteenth day of treatment, the animals rapidly regained 10% of their body weight in 2 days to match that of the controls, and signs of emaciation disappeared.

DISCUSSION

The production of gastric ulcers in fasting rats by use of D1C confirms the work of Robert and Nezamis (17), and illustrates the efficacy of the method.

The failure of pantothenyl alcohol to prevent D1C-induced gastric ulcers, in contrast to its apparent accelerating effect on healing, may be due to the overwhelming action of the massive doses of steroid during the ulcer induction period.

The sustained depression of body weight, following the 4 days ulcer induction period, corresponds to the presence of D1C deposits in the subcutaneous tissue, and probably prevented a more rapid spontaneous ulcer healing.

The mechanism of pantothenyl alcohol in accelerating the healing of experimental gastric ulcers is obscure. Zucker (22) has suggested as the etiology of the experimental rat duodenal ulcer during pantothenic acid deficiency, that the target area or duodenal mucosa becomes sensitized to the ulcerogenic effect of the adrenocortical steroids. In D1C-induced gastric ulcers, existing levels of mucosal pantothenate may be less adequate in maintaining and restoring cellular integrity. Pantothenyl alcohol may aid in the maintenance and reparative processes of the gastric mucosa by providing a greater level of tissue pantothenate.

The emaciation and the alopecia observed in the rats on the ninth day of treatment is unusual in that the effects appeared as a result of the pantothenyl alcohol treatment. Unna and Greslin (23) found no toxicity when 200 mg. of calcium pantothenate was fed daily to rats for a period of over 6 months. Their average dosage was roughly three times that of pantothenyl alcohol used in the present experiment. The observed toxic effects further emphasize the role of pantothenic acid under stress conditions.

SUMMARY

1. Gastric ulcers were experimentally produced in fasted female rats by subcutaneous administration of D1C for 4 days.

2. Pantothenyl alcohol given during the induction period did not significantly alter the development of D1C-induced gastric ulcers.

3. Pantothenyl alcohol markedly accelerated the healing of gastric ulcers when given during the recovery period. Complete healing was observed by 16 days in the pantothenyl alcohol group, but required a longer period in the control animals.

4. Pantothenyl alcohol-treated animals showed obvious evidence of toxicity, characterized by emaciation and alopecia on the ninth day of treatment, which disappeared upon withdrawal of the drug.

5. The possible role of pantothenyl alcohol, relative to its effect on D1C-induced gastric ulcers, is discussed.

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