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Toxicity and Pharmacology of SN 13592.* An Analogue of Phenyl Pantothenone.

HERON O. SINGHER, NATHAN MILLMAN, AND MARY RICE BOSWORTH.
From the Ortho Research Foundation, Raritan, N.J.

The oral antimalarial activity of phenyl pantothenone[1] led to the preparation of compounds which might be considered as thio analogues.[2] One of these was SN 13592,[3] (-)-α,γ-dihydroxy-β,β-dimethyl-N-(2-(phenyl-mercapto-)-ethyl)-butyramide. This compound was examined for trichomonicidal activity.[4] Since high *in vitro* activity was demonstrated, parallel studies of toxicity and systemic effects of this substance were conducted, as representative of the type of phenomena which might be shown by a series of similar phenyl pantothenone analogues. The toxicity tests, for the most part, were based on those described in the *Survey of Antimalarial Drugs*,[3] with minor modifications. The tests are referred to by the designations given them in that publication.

Acute toxicity. Pantothenic acid has an extremely low oral toxicity for the rat. The lethal dose is greater than 10 g/kg.[5] SN 13592 is characterized by a similar low toxicity, being of the order of 5 g/kg. The administration of larger doses by means of the stomach tube was difficult because of the low solubility of the compound. Death at toxic levels of calcium pantothenate is the result of respiratory failure, although lower doses do not seem to affect the respiratory system.[6] With SN 13592, all doses above 500 mg/kg were attended by marked respiratory difficulty, which usually disappeared within 1-2 hours in surviving animals. This respiratory effect was the subject of further study, as described under "systemic effects."

While acute intravenous toxicity was not investigated, it was noted that in one case a dog was infused with a total of 2.6 g (397 mg/kg) over a period of 5 hours without fatal result.

Chronic toxicity. Tests were performed to determine the chronic toxicity for several species of animals. An 11-day rat test was conducted according to the procedure of Test 1-U,[3] with the deviations in weight gains between experimental and control animals as the criterion. During the experimental period, groups of rats receiving 1/16, 1/8, or 3/16 of the lethal dose daily averaged a weight gain similar to that of control rats fed the vehicle alone, and also similar to that of control rats fed the same fraction of the lethal dose of a reference compound, quinine. As shown in Table I, there were a few deaths, but it is doubtful whether these were attributable to the drugs since they also occur with the reference drug.

Histological examination of major organs of these rats, including the intestinal tract, revealed no significant pathological changes.

A 7-day mouse test (Test 1-B) gave similar results as far as weight gains were concerned. Groups at 45, 90, and 270 mg/kg/day exhibited average weight gains comparable to the control animals, but mortality was much higher at daily doses greater than 270 mg/kg.

For studies with the chick Test 1-A was used. The maximum tolerated dose (that daily dose at which the final weight is approximately equal to the starting weight) was determined to be 4000 mg/kg/day for the 4 days of the test. However, at 500 mg/kg/day the chicks gained as much weight as the controls, and as much as the group fed 50/kg/day of the reference drug quinine. It is interesting to note the variation in weight

* The Survey Number, SN 13592, designates this compound in the *Survey of Antimalarial Drugs.*

1 Woolley, D. W., and Collyer, M. L., *J. Biol. Chem.*, 1945, **159**, 263.

2 Senear, A. E., Rapport, M. M., and Koepfli, J. B., *J. Biol. Chem.*, 1947, **167**, 229.

3 Wiselogle, F. Y., *A Survey of Antimalarial Drugs*, Edwards, Ann Arbor, 1946.

4 Johnson, G., and Kupferberg, A. B., in press.

5 Molitor, H., *Fed. Proc.*, 1942, **1**, 309.

6 Unna, K., and Greslin, G., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 311.

TABLE I.
Chronic Toxicity of SN 13592—Test 1-U.

Group	No. of rats	Compound	Fraction of lethal dose daily	Total mg/kg (11 days)	No. of animals surviving	Avg wt gain (g/11 days)
A	6	Control	—	—	6	49.8
B	6	Quinine	1/16	308	5	45.4
C	6	"	1/8	616	6	50.0
D	6	"	3/16	924	6	49.0
E	5	SN 13592	1/16	3432	4	42.5
F	6	"	1/8	6875	6	60.3
G	6	"	3/16	10307	5	40.0

TABLE II.
Chronic Toxicity of SN 13592—Test 1-A. Five chicks in each group.

Group	Compound	Dose mg/kg/day	No. of chicks surviving	Avg wt change (g/4 days)
A	Vehicle	—	5	+22.4
B	Quinine	350	4	+16.2
C	"	200	5	+25.8
D	"	100	5	+25.8
E	"	50	5	+28.0
F	SN 13592	4000	4	— 1.2
G	"	2000	4	+11.0
H	"	500	5	+28.8
I	"	100	5	+20.8

gain with dosage, as shown in Table II.

Over a period of almost 6 weeks, one Rhesus monkey received a total of 3.6 g orally, in doses beginning at 10 mg twice a day, and progressively doubling each week. During this time no digestive or physiological disturbances were observed other than a hematological change. The blood picture altered markedly with the appearance of a decrease in red blood cells and a concomitant decrease in hemoglobin. Fig. 1 describes the rather rapid induction of this anemia, and the almost total recovery during the 39 days after

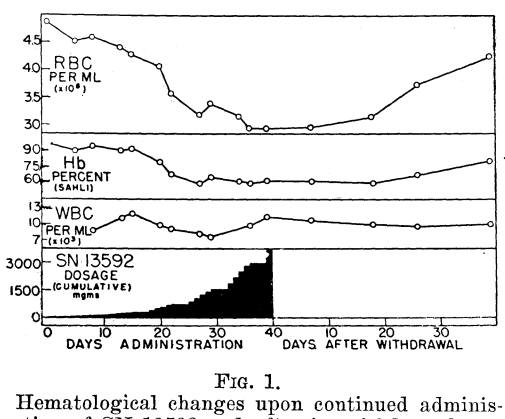

FIG. 1.
Hematological changes upon continued administration of SN 13592 and after its withdrawal.

cessation of SN 13592 administration. An attempt to induce the same type of anemia in rabbits and rats was unsuccessful.

Local Effects. Ten per cent solutions of calcium pantothenate have been found to have no irritating effect on the conjunctival mucosa of the rabbit.[6] In the present study no gross or microscopic pathology was observed in the vaginal tissues of animals in which SN 13592 was applied to the vaginal mucosa in saline. In 20 treatments over a 10-day period 4 rats received a total of 20 mg each, 2 guinea pigs a total of 50 mg each, and 2 rabbits a total of 200 mg each.

Systemic Effects. The respiratory effect observed upon oral and intravenous administration of SN 13592 led to preliminary studies of its effect on respiration, circulation and smooth musculature. Intravenous administration evoked inconsistent variations in blood pressure as determined by carotid cannulation and a mercury manometer. Doses as high as 50 mg/kg in the cat induced only relatively minor changes in blood pressure. The heart rate showed similar minor variations in this range of drug administration.

The effect of intravenous SN 13592 on respiration is almost immediate, although of

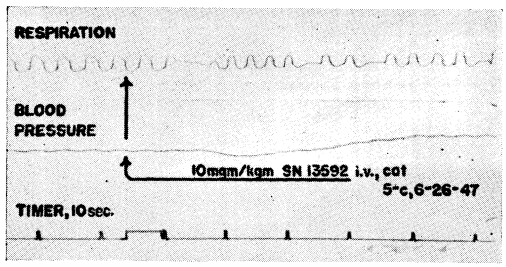

FIG. 2.
Blood pressure and respiratory system effects of SN 13592.

short duration. Respiration became shallower and erratic in the cat after intravenous injection of doses greater than 10 mg/kg (Fig. 2). In some cases it ceased entirely for an interval of a few seconds. Control injections of the vehicle alone failed to produce this response.

It was also found that the compound had a small effect on smooth musculature. *In vitro* intestinal strip preparations were slightly stimulated by 1:2000 dilutions. Ten mg/kg of SN 13592 administered intravenously in the cat enhanced uterine contractility.

Summary. SN 13592 is characterized by an extremely low acute and chronic oral toxicity in several species. Local irritation effects are absent even upon repeated administration. Continued oral feeding of the drug induces an anemia which gradually disappears after the drug is withdrawn. Although there were no consistent effects on blood pressure and heart rate, a respiratory effect was noted. Smooth muscle was generally stimulated by this compound.

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Chemotherapy of Bacteria-Free *Trichomonas vaginalis*. III. Action of Analogues of Pantothenic Acid.*

GARTH JOHNSON AND ALFRED B. KUPFERBERG.
From the Division of Microbiology, Ortho Research Foundation, Raritan, N.J.

The recent demonstration by Kupferberg *et al.*[1] that *Trichomonas vaginalis* requires pantothenic acid motivated this study of the inhibitory effects of pantothenate analogues.

Materials and Methods. The effect of a variety of compounds as growth inhibitors was first explored using strain No. 2 of bacteria-free *Trichomonas vaginalis*.[2] Refined studies were later run using 7 additional pure cultures of *T. vaginalis*. In addition 3 strains of *T. foetus*† and one strain of *T. gallinae* were explored using a single highly active compound.

One ml of an appropriate stock dilution of each compound was added to 9 ml of Simplified Trypticase-Serum medium[1] containing 3.2 μg of added calcium pantothenate per 10 ml of final medium. The solvent used was either water or 95% ethanol. In no case where alcohol was used as a solvent was more than 0.1 ml of alcohol introduced into 10 ml of culture medium. In such instances a control culture containing a like amount of alcohol without drug was carried. The inoculum consisted of 0.05 ml of a 36- to 40-hour culture containing 100,000 trichomonads. The cultures were incubated at 37°C and

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1 Kupferberg, A. B., Johnson, G., and Sprince, H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 304.

2 Johnson, G., Trussell, M., and John F., *Science*, 1945, **102**, 126.

† The three strains of *T. foetus* used in this study were supplied by Dr. B. B. Morgan, University of Wisconsin.