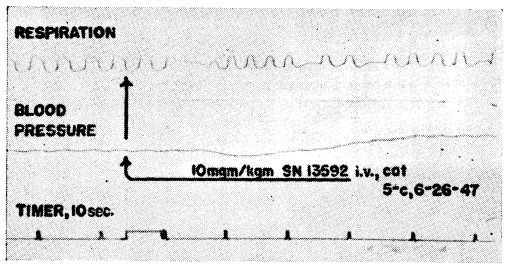

FIG. 2.
Blood pressure and respiratory system effects of SN 13592.

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 Continued oral feeding of the drug induces an anemia which gradually disappears after the drug is withdrawn.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.

TABLE I.
Effect of Analogues of Pantothenic Acid upon Strain No. 2 *Trichomonas vaginalis*.

No.		Compound	Observations on 9th day: Lethal at one part in: × 1000	Observations on 9th day: Complete inhibition at one part in: × 1000
1.	SN 13593	(+)-α-γ-Dihydroxy-β,β-dimethyl-N-(2-(phenylsulfinyl)-ethyl)-butyramide	8	16
2.		d(+)-(pantoyltauryl)-p-anisidide	10	20
3.	SN 12610	N-(2-Benzylethyl)-α-γ-dihydroxy-β,β-dimethyl-butyramide. Prepared from natural (—) lactone	400	500
4.	SN 13592	(+)-α-γ-Dihydroxy-β,β-dimethyl-N-(2-(phenylmercapto)-ethyl)-butyramide	750	—
5.	”	Decolorized by norite	800	—
6.	”	Crystalline fraction	400	
7.	”	Fraction boiling up to 55°C at 10^{-4} mm Hg	400	
8.	”	Fraction boiling 55-155°C at 10^{-4} mm Hg	400	
9.	”	Fraction boiling 155-190°C at 10^{-4} mm Hg	400	

Compounds 1 and 4 were received from Dr. J. B. Koepfli, California Institute of Technology. No. 2 was supplied by Dr. J. P. English, American Cyanamide Co. No. 3 was supplied by Dr. R. E. Lutz, University of Virginia. Nos. 5, 6, 7, 8, and 9 were prepared by Dr. Wm. Oroshnik and Mr. R. A. Mallory in our own Division of Organic Chemistry.

TABLE II.
Inhibition of Eight Strains of *T. vaginalis* by SN 13592. (Decolorized by Norite.)

Strain No.	Effective dilutions one part in: × 100,000	Not effective at one part in: × 100,000
1	10	11.2
2	8	—
3	14	16
4	7	9
5	9	10
6	6	7
7	2	3
9	9	10

Strain No. 1 was isolated in 1939.[3]
Strains No. 2-No. 7 were isolated in 1945.[2]
Strain No. 9 was isolated in 1947 from a case of acute vaginal trichomoniasis with the assistance of C. E. Folsome, M.D.

observed microscopically after 3, 6, and 9 days.

The data presented in Table I summarize the results obtained with a series of compounds using strain No. 2 as the test organism. Table II shows the comparative sensitivity of 8 strains of *T. vaginalis*.

The data in Table II reveal a sevenfold variation in sensitivity to SN 13592, ranging from 1:200,000 to 1:1,400,000.

The following compounds were found to have little or no activity at a concentration of 1:10,000:

1. dl-N-Pantoylisoamylamine
2. dl-N-Pantoyl-N-butylamine
3. dl-N-Pantoylethanolamine
4. d(+)-Pantoyltauryl-p-nitroanilide
5. d(+)-Pantoyltauryl-p-benzylamide
6. 2-(d(+)-Pantoyltauryl)-amino-5-Chloropyridine
7. d(+)-Pantoyltauryl-p-Carboxyanilide
8. dl-N^4-Pantoyltauryl-Sulfanilamide

Compounds 1, 2, and 3 were supplied by Dr. Wm. Shrive, University of Texas. Compounds 4 through 8 were received from Dr. J. P. English, American Cyanamide Company.

Table III shows that the sensitivity of *T. foetus* to the pantothenate analogue is in the same range as that of *T. vaginalis*. The single strain of *T. gallinae* appears to be slightly more sensitive than most strains of *T. vaginalis*. As noted in a previous publication[1] these other trichomonad species grow

TABLE III.
Effect of Norite Treated SN 13592 on *T. foetus* and *T. gallinae*.

Organism	No. surviving cells at one part in × 10,000
T. foetus 3P	30
” BR	40
” AN	50
T. gallinae	100

[3] Trussell, R. E., and Plass, E. D., *Am. J. Obst. and Gynec.*, 1940, **40**, 883.

luxuriantly in the same Simplified Trypticase-Serum medium.

In an effort to determine whether the inhibitory effect of SN 13592 against *T. vaginalis* could be reversed with added Ca-pantothenate, 10 ml of media containing 1:200,000 drug with and without 320 μg of Ca-pantothenate were inoculated. No surviving cells were found in the absence of the Ca-pantothenate. With the added pantothenate the experimental population was equal to that in the control. It is thus suggested that, as with other microörganisms, the analogue of pantothenic acid acts in competition with the natural compound.

Due to its high level of *in vitro* activity it was thought worthwhile to submit SN 13592 to a study of its possible therapeutic activity in infected monkeys. Studies on the pharmacology of the compound by Singher, Millman, and Bosworth[4] made possible a study of the therapeutic effect in cases of human vaginal trichomoniasis.‡

Three monkeys, each weighing 5.5 kg, were successfully infected with a recently isolated strain (No. 9) of *T. vaginalis* which was obtained from a patient with an acute vaginitis.

Monkey No. 1 received 8 doses of 5.5 mg intravenously, followed by 24 oral doses of 300 mg administered twice daily. She remained infected and was therefore treated vaginally. Seven 300 mg doses were administered per vagina. Subsequent microscopic examination of the vaginal fluids revealed a persisting infection.

Monkey No. 2 received 9 oral doses of 275 mg given once daily. The infection persisted in spite of treatment. Seven vaginal doses of 300 mg were then administered without elimination of the infection.

Monkey No. 3 was given a long series of graded oral doses, given twice daily as follows: 10 doses each of 10 mg, 20 mg, 40 mg, and 80 mg, followed by 9 doses of 160 mg, and 2 320-mg doses. The total drug administered amounted to 3.6 g. As in the other 2 monkeys, the infection was not eliminated.

In a series of 6 clinical cases of *Trichomonas vaginalis* vaginitis, a course of vaginal tablets containing 1 mg of SN 13592 was administered twice daily for 2 weeks. All 6 patients retained their infections in the face of the estimated 1:3000 dilution of the drug.

The failure of this analogue of pantothenic acid to eradicate vaginal trichomonad infections is referable to the pantothenic acid content of the blood serum. Denko, Grundy, and Porter[5] demonstrated an average blood concentration of 33 μg per 100 ml. McIlwain and Hawking[6] reported a sensitivity of staphylococci and streptococci to pantoyltaurine. Mice infected with these bacteria were not protected by pantoyltaurine. However, rats which have a lower blood level of pantothenic acid survived such infections when given a protective dose of pantoyltaurine. Woolley and White[7] were unable to produce a pantothenate deficiency in mice by long continued administration of the same compound.

Conclusions. 1. The *in vitro* effectivity of several analogues of pantothenic acid has been demonstrated against *T. vaginalis*. 2. Wide variations in susceptibility of different strains to SN 13592 were found. 3. *T. foetus* and *T. gallinae* were found highly sensitive to this compound. 4. The *in vitro* activity of SN 13592 was reversed by the addition of calcium pantothenate. 5. *In vivo* studies in monkeys and in human beings demonstrated failure of SN 13592 to eradicate the infection.

‡ The authors are indebted to C. E. Folsome, M.D., for these clinical trials.

4 Singher, H. O., Millman, N., and Bosworth, M. R., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 388.

5 Denko, W. D., Grundy, W. E., and Porter, J. W., *Arch. Biochem.*, 1947, **13**, 481.

6 McIlwain, H., and Hawking, F., *Lancet*, 1943, **1**, 459.

7 Woolley, D. W., and White, A. G. C., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 106.