Antituberculosis Activity of Vinactane

Introduction. In previous report[1], a description was given of a new antibiotic produced by a hitherto unknown species of actinomyces, *Streptomyces vinaceus*. This antibiotic substance, referred to as Vinactane, possesses antibacterial as well as pronounced antituberculosis activity in experimental infections. It was subsequently found that Vinactane was identical to the later discovered viomycin[2].

Vinactane is a strongly basic polypeptide; the empirical formula for the free base is $C_{17-18}H_{31-35}N_9O_8$. Hydrolysis experiments have suggested that Vinactane is a peptide possibly of cyclic nature. It is recovered during the isolation process as a hydrochloride[3]. It is readily soluble in water. At pH 2·0 to 7·0 it is very stable in aqueous solutions held at room temperature for prolonged periods and can be heated for 10 min at 100°C without loss of antibiotic activity.

Table I
Bacteriostatic Activity of Vinactane Sulfate

Test Organism	Smallest concentration completely inhibiting growth (γ/ml)	
	Vinactane	Streptomycin
Staph. aureus	65	4
Staph. aureus (resistant to streptomycin)	100	2000
Strep. pyogenes	16	10
Bacillus subtilis	16	200
Bacillus anthracis	24	200
Diplo. pneumoniae	>200	2
E. coli	75	20
Kleb. pneumoniae	23	2
Pseud. aeruginosa	375	50
Salm. schottmuelleri	185	100
Shig. paradysenteriae	185	10
Br. abortus	31	50
Myco. tuberc. hominis (H37Rv)	0·6	0·6

Not only has considerable chemical work on Vinactane[3] suggested that this antibiotic is identical with viomycin, but bacterial spectra studies have confirmed this view. Furthermore, cross-resistance experiments have shown that a strain of *Staph. aureus* made resistant to either antibiotic was equally resistant to both substances.

Experimental. In vitro Activity of Vinactane Sulfate. The bacteriostatic endpoints obtained with a variety of test organisms, including several species of mycobacteria, are shown in Table 1. The method used consisted of preparing varying dilutions of the antibiotic in suitable liquid media and determining the highest dilution capable of completely inhibiting the growth of the organisms. For comparative purposes, streptomycin was similarly tested.

From the results of the *in vitro* tests, it is apparent that Vinactane is particularly active against the mycobacteria, moderately active against the usual Gram-positive organisms, and of lesser activity against a variety of Gram-negative bacteria. Although it possesses a range of activity similar to that of streptomycin, Vinactane is capable of inhibiting the growth of streptomycin-resistant strains of *Staph. aureus* and H37Rv to the same degree as demonstrated with the parent, sensitive strains. Similarly, PAS and isoniazid-resistant strains of the H37Rv culture were sensitive to the action of Vinactane.

Toxicity of Vinactane.—A dose of 1000 mg/kg, administered subcutaneously, once daily for 21 consecutive days, was well tolerated by mice and was without apparent toxic effects. Guinea pigs inoculated intramuscularly with daily doses of 20 mg/kg for 60 consecutive days demonstrated no ill effects. The approximate subcutaneous, intraperitoneal and intravenous acute LD_{50} values for mice are 35, 25, and 4 mg/kg respectively.

Chemotherapeutic Activity in Tuberculous Mice.—a) *Graded-dose experiment:* CFl mice, weighing between 15 and 20 g each, were infected intravenously with 0·5 ml of a 1:10 dilution of H37Rv grown for 7 days in KIRCHNER's liquid medium containing 0·03% Triton A-20 and 0·2% bovine serum albumin. Treatment with Vinactane sulfate was started immediately after infection with varying levels of the antibiotic administered subcutaneously, and therapy was continued for 21 consecutive days. Surviving animals were sacrificed on the 32nd day after infection. Two criteria were used for the evaluation of activity: percent survival on the 32nd day and the T50 value which represents the calculated survival time (days) of 50% of the mice, obtained by plotting the cumulative percentage dead on a probability scale against time on an arithmetic scale[1]. The results of this experiment, shown in Table 2, indicate that Vinactane sulfate, at doses greater than 0·5 mg and administered daily for 21 days, is capable of exerting a significant degree of antituberculosis activity. Streptomycin however, on a dosage basis proved more effective than Vinactane. HOBBY and co-workers[2] found that doses of 0·5 mg or more of viomycin daily were sufficient to increase significantly the average survival time and the percentage survival of infected animals.

Table II
Antituberculosis Activity in Mice of Vinactane Sulfate Administered Subcutaneously at Varying Dose Levels

Antibiotic	Daily Dose (mg)	% Survivors	T50 Value (days)
Vinactane Sulfate	2·0	100	> 32·0
	1·0	80	> 32·0
	0·5	10	22·0
Streptomycin Sulfate	2·0	100	> 32·0
	1·0	100	> 32·0
	0·5	90	> 32·0
Controls (no treatment)	–	0	17·0

(b) *Effect of delayed therapy.* In this experiment, the administration of the antibiotic was delayed for 0, 3, 6, 9, 12, and 15 days after infection or treatment main-

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Table III

Antituberculosis Activity in Guinea Pigs of Vinactane Sulfate at Varying Dose Levels.
Treatment Started: 28th day, post-infection. *Duration of Treatment:* 60 days.

Dose Vinactane Administered	Number of Animals	Average Gross Involvement				Average Total Involvement
		Spleen	Lungs	Liver	Site of Inoculation	
10·0 mg	11	10·0	6·4	7·7	3·6	27·7
5·0 mg	15	6·3	10·0	6·7	4·7	27·7
2·0 mg	12	18·8	10·8	18·8	8·3	56·7
Pretreatment Controls*	5	26·0	10·0	16·0	10·0	62·0
Untreated Controls (88th day)	13	19·2	20·8	23·5	10·0	73·5

* The pre-treatment controls were sacrificed at the commencement of therapy (28th day, post-infection) in order to establish the extent of tuberculous involvement.

tained for 21 consecutive days. In the 38th day, post-infection, all surviving mice were sacrificed. According to the results obtained, therapy with 1·0 mg Vinactane can be delayed for nine days after infection and yet exert a strong antituberculosis activity. At the 0·5 mg dose level, treatment with the antibiotic can be delayed for two days beyond the time of infection and still yield excellent activity.

Chemotherapeutic Activity in Tuberculous Guinea Pigs. Female guinea pigs, approximately 500 g in weight, were infected subcutaneously in the groin with 1·0 ml amounts of a 1:50 dilution of a 7-day old culture of H37Rv. Twentyseven days after receiving the infecting dose, the animals were tuberculin tested (intracutaneous injection of 0·1 ml of 1% "O.T.") and all responded with a strong positive reaction. Groups of 12 animals each were, on the 28th day following infection, treated with 10·0, 5·0 and 2·0 mg Vinactane sulfate, administered intramuscularly once daily for 60 days. The normal animal diet was supplemented with greens and ascorbic acid, the latter added to the drinking water (0·5 mg/ml) on alternate days. On the last day of therapy (88th day, post-infection) all animals were sacrificed. The gross organ involvement was determined according to the method described by FELDMAN and KARLSON[1]. Maximal values of 40, 70, and 100 respectively were assigned to animals with slight, moderate, and extensive degrees of total organ involvement. The results of this experiment are shown in Table 3 and indicate that Vinactane is capable of exerting appreciable anti-tuberculosis activity in guinea pigs at doses of 2·0 mg per day.

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Zusammenfassung

Vinactan, eine antibiotische Substanz, isoliert von *Streptomyces vinaceus*, besitzt bedeutende antituberkulöse Aktivität in infizierten Mäusen und Meerschweinchen. Erheblicher Schutzeffekt in tuberkulösen Mäusen wurde beobachtet, wenn die Therapie nicht sofort, sondern erst 9 Tage nach der Infektion einsetzte und die Behandlung mit 1 mg täglich während 21 Tagen durchgeführt wurde. In tuberkulösen Meerschweinchen, die mit 5 mg Vinactan täglich subkutan während 60 Tagen behandelt wurden, war die Entwicklung tuberkulöser Infektion in Milz, Lunge und Leber erheblich vermindert.

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Occurrence of an Enzyme Acting on Xanthopterin-B in *Bombyx mori*

A pterin like pigment, xanthopterin-B (the suffix "B" coming from *Bombyx*)[1] is found in the integument of larvae of the mutant, "lemon" (*lem*)[2] and "yellow lethal" (*lem*^{l})[3], of the silkworm, characterized by the yellow colour of their skin. It is also found in the wings of the yellow butterflies, *Eurema* and *Colias*. Both in chemical properties and biological activity, xanthopterin-B has a similarity to xanthopterin and to folic acid[4]. However, xanthopterin-B has not been isolated in a pure form for the determination of its structural formula. It shows a strong yellow fluorescence and like folic acid is very unstable to light, especially in acid solution. The relation between xanthopterin-B, uric acid and melanin in the skin of several mutants of the silkworm has been studied from the stand-point of biochemical genetics[5].

Using 5% sodium citrate as a solvent, xanthopterin-B in the tissue extract of the *lem* larvae separated into two components on a paper chromatogram. We designated the two components as xanthopterin-B_1 (*Rf* value, 0·27) and xanthopterin-B_2 (*Rf* value, 0·32)[4]. The two spots, however, overlapped when a common solvent such as butanol-acetic acid-water (4:1:1) was used (*Rf* value, 0·45). Aside from sodium citrate the following solvents were also useful for differentiating the two components: sodium acetate, sodium chloride, ammonium chloride and urea (all 5% solutions). Both xanthopterin-B_1 and -B_2 show yellow fluorescence, but the former fluorescents much more strongly and is also more unstable toward some chemical treatments. In the integument of the *lem* larva four pterins or pterin like pigments, leucopterin, leucopterin-B ("isofluorescyanine [fluorescyanine B]"[6], or probably identical with isoxanthopteria[7])

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