compound, recrystallization, regeneration of the citral, and distillation at reduced pressure. The purified material was then tested against bacteria, yeasts, and molds (Tables I, II, and III).

The purified citral shows activity against all the gram-positive bacteria at 500 ppm[13] and one of the gram-negative bacteria. The effect drops off somewhat at 250 ppm as indicated in Table I. However, when one separates the citral into its components (neral and geranial, geometric isomers) by preparative gas-liquid chromatography and tests these under the same conditions, both are essentially inactive against all of the bacteria tested. To eliminate the possibility of a synergistic effect, a 50:50 mixture of neral and geranial was prepared and tested under identical conditions. The results were the same as those obtained with the pure components.

A similar experiment was conducted with representative strains of yeasts and molds and the same pattern emerged (Tables II and III) but the results were not quite as dramatic.

The activity of citral against the species of yeasts does not begin to drop off until a concentration of 62.5 ppm is reached. In contrast, neral begins to be ineffective at 250 ppm. It is of interest to note that geranial is somewhat different than neral since total inhibition of yeast growth is observed at 250 ppm. There does not appear to be any difference between geranial and neral with respect to bacteria and molds.

The effect of concentration of citral against various species of molds is summarized in Table III. At 125 ppm citral begins to lose effectiveness against *Aspergillus oryzae*, *A. niger*, and *Rhizopus senti*. At 125 ppm, it is still effective against the 4 remaining molds. Geranial was found to be ineffective at 500 ppm against 4 of the 7 molds, whereas neral was ineffective against 3 of the 7 molds. It becomes quite obvious that an impurity exists in the 'pure' citral.

An interesting observation is found in the action of a 50:50 mixture of neral and geranial against *Penicillium chrysogenum*. Neither neral nor geranial alone at 500 ppm was effective against this species of mold; yet, a combination of 250 ppm of each showed activity. It appears that this is one instance of synergism. Several examples of synergistic effects are reported, e.g., ZIBITSKER[11].

These data, then, cast doubt on the antimicrobial activity of citral as reported in the literature. The 'pure' citral used in these experiments was as pure or purer than that used by the previous workers. As can be seen, the 'pure' citral showed considerable activity but the isolated components failed to substantiate the previous findings.

In view of these data one must conclude that an impurity exists in the 'pure' citral which has substantial activity and which also is carried through the purification process. An examination of an analytical gas chromatogram of the purified citral showed it to be approximately 98% citral. Work is now in progress to identify the impurities and determine which of these have antimicrobial activity.

Zusammenfassung. Bestätigung früherer Feststellungen, dass Citral eine antibakterielle Aktivität besitzt. Da Neral und Geranial (allein oder zusammen) jedoch wesentlich weniger aktiv sind, muss für die Citralaktivität eine bisher nicht identifizierte Substanz verantwortlich sein.

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Agricultural Research Service,
U.S. Department of Agriculture,
Albany (California 94710, USA), 7 December 1970.

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Macrophage Activity of Thymectomized Mice Infected with *Leishmania donovani*

Although the importance of the thymus in cell-mediated immunity and in the production of some humoral antibodies is established[1], there are several contradictory reports on the activity of macrophages in thymectomized animals[2-6].

In mice experimentally infected with the intracellular protozoan parasite of macrophages, *Leishmania donovani*, the number of parasites in the liver and spleen reach a peak after which numbers of parasites in these organs decline and the infection becomes chronic[7]. Thus, this infection in mice could be a good system to determine the effect of thymectomy on macrophage activity in vivo and in vitro.

Balb/c mice, thymectomized[8] or sham-thymectomized within 24 h of birth, were used in the in vivo studies. Each mouse was injected i.v. with 10^7 amastigotes[9] of the 3K strain of *L. donovani*[10] in 0.2 ml of infected hamster spleen suspension 25–30 days following thymectomy. Mice from each group were sacrificed 1 h after infection to determine the initial uptake of parasites by spleen and liver, and at 1, 8, 16 and 21 days to determine rate of growth or suppression. The median level of infection in livers and spleens of 5–8 mice of each group was determined by a method of touch preparation[10]. At necropsy, thymectomized mice were examined macroscopically and microscopically to determine whether thymectomy was complete. Mice with remains of thymus were not included in the results.

Peritoneal macrophages from 25- to 30-day-old thymectomized, sham thymectomized and intact mice were

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harvested from 3 to 7 mice of each group 48 h after i.p. injection of 2 ml of sterile trypticase soy broth. From 1×10^7 to 2×10^7 macrophages were explanted in plastic petri dishes, each containing 8 coverslips in a manner similar to that described by TAKEYA et al.[6]. Each petri dish was inoculated 24 h later with a suspension of tissue containing amastigotes of *L. donovani* from the spleen of an infected hamster in a ratio of about 10 parasites/macrophage. After 1 h, the cultures were washed to remove uningested parasites and new medium was added to all cultures.

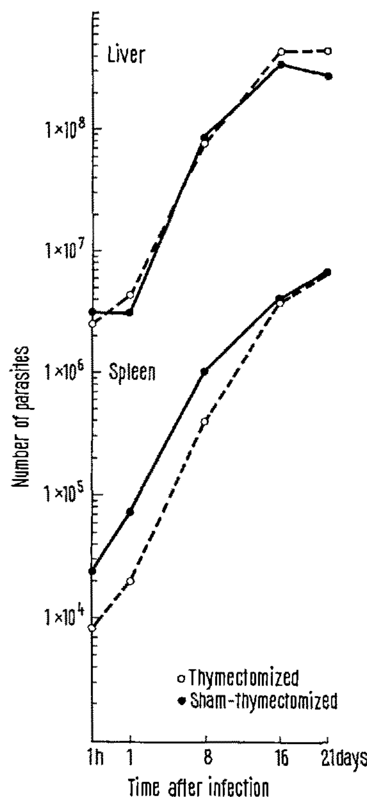
Macrophage activity was assessed by determining the proportion of parasitized cells and by calculating the mean number of parasites/macrophage after examining 100 randomly selected cells on Giemsa-stained coverslips removed from petri dishes 1, 24, 48 and 72 h after inoculation of the parasites.

When the parasite burdens of the livers and spleens of both groups of mice were compared 1 h after infection, no significant differences between groups in the number of parasites in these organs were observed. The rates of reproduction of the parasites in the 2 groups of mice were also similar (Figure). For example, the median parasite burden in spleens of thymectomized mice increased from 1.96×10^4 parasites 1 day after infection to 3.94×10^5 parasites on the 8th day, a 20.1-fold increase. This increase does not differ significantly from the one in the sham-thymectomized mice, i.e., from 7.22×10^4 to 1.00×10^6, a 13.9-fold increase. Similarly, between 16 and 21 days post-infection, the median parasite burden of spleens of thymectomized and sham-thymectomized mice increased 1.8 and 1.7 times, respectively. Increases in the median numbers of parasites in the liver of groups of thymectomized and sham-thymectomized mice were not significantly different when compared to each other at any time of sampling. These results indicate that the rate of growth or intracellular suppression of the amastigote in vivo is not modified by thymectomy.

In vitro the proportion of infected macrophages from thymectomized, sham-thymectomized and intact mice was 93, 95, and 95% with 6.1, 5.9 and 6.1 parasites per macrophage, respectively, 1 h after infection of the culture. The results in samples taken at 24 and 48 h were equally similar and by 72 h the respective proportion of infected macrophages were 60, 70 and 58% with 1.2, 1.7 and 1.6 parasites per macrophage (Table). Thus, there were no significant differences between thymectomized or either control group either 1 h after the infection or in the rate of growth or suppression of parasites by macrophages after 24, 48 or 72 h.

These results indicate that thymectomy does not modify the susceptibility of Balb/c mice to *L. donovani* and contrast sharply with the impaired capacity of thymectomized animals to overcome infection with *Plasmodium berghei* or *Trypanosoma cruzi*[3,11,12]. Resistance to the latter 2 probably is impaired by delayed or decreased production of specific humoral antibodies which are known to be functional in the control of these infections[13,14], whereas antibodies do not protect a host experimentally infected with *Leishmania*[15] nor influence the activity of macrophages on amastigotes in vitro[16].

Although some investigators indicated increased clearance of carbon by thymectomized animals[2,3], others were unable to detect differences in phagocytosis of colloidal gold[4] or carbon[5] by thymectomized or intact animals, or of bacteria in vitro[6]. Our results indicate that there is no significant difference in phagocytosis of amastigotes



Number of parasites in livers and spleens of thymectomized and control mice at various times after infection with amastigotes of *Leishmania donovani*.

In vitro phagocytosis of amastigotes of *L. donovani* by macrophages from thymectomized, sham-thymectomized and normal mice

Time (h)	After infection	Treatment		
		Thymectomized	Sham-thymectomized	Normal
1	Macrophages infected (%)	93	95	95
	Mean No. of parasites per cell	6.1	5.9	6.1
24	Macrophages infected (%)	82	88	86
	Mean No. of parasites per cell	3.5	3.6	2.7
48	Macrophages infected (%)	73	76	61
	Mean No. of parasites per cell	2.4	3.0	2.1
72	Macrophages infected (%)	60	70	58
	Mean No. of parasites per cell	1.2	1.7	1.6

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in thymectomized mice compared to normal mice at 1 h after infection.

In contrast to the results reported by TAKEYA et al.[6] with *Listeria monocytogenes* in vitro, we did not observe enhancement of intracellular killing of amastigotes in macrophages derived from thymectomized mice. These experiments establish that macrophage activity in experimental leishmaniasis is thymus independent. Such activity could be inherent to the macrophages or depend on immunologically competent tissues formed early in the ontogenic development of the host[17].

Resumen. Fagocitosis y destrucción de *Leishmania donovani* por macrófagos in vivo o in vitro es igual en macrofagos de ratones timectomizados e infectados que en macrófagos de ratones no timectomizados e infectados. Se concluye que la resistencia natural contra infección con *L. donovani* no es disminuida por la timectomía. Además, los datos indican que la actividad de los macrófagos es independiente del timo.

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STUDIORUM PROGRESSUS

Structures of the Venturicidins A and B

The venturicidins A and B, two antifungal antibiotics from *Streptomyces aureofaciens*, strain Tü 342, are a 3-*O*-carbamyl-2-deoxy-D-rhamnoside and a 2-deoxy-D-rhamnoside, respectively, of an unknown aglycone[1]. Although the aglycone itself was decomposed during solvolytic reactions, spectroscopic coincidences strongly suggested the identity of the two aglycones. Additional evidence for this identity, the isolation of several common degradation products from the aglycone part, will be given in this paper.

The structure of venturicidin A was determined by restricted use of conventional organic chemistry methods, by an extensive application of NMR- and mass-spectrometry, and by a three-dimensional X-ray analysis of a heavy-atom derivative, the mono-*p*-iodobenzenesulfonate V.

X-ray analysis. The derivative V was prepared by treatment of venturicidin A with *p*-iodobenzenesulfonyl chloride in dry pyridine at room temperature. The crystals were grown from a water-acetone solution by slow cooling. Crystal data: $C_{47}H_{70}INO_{13}S \cdot H_2O$; *ortho*rhombic disphenoidal with $a = 5.76 \pm 0.05$, $b = 31.4 \pm 0.2$, $c = 31.3 \pm 0.2$ Å; $V = 5661$ Å^3; $F(000) = 2168$. Space group $P2_12_12_1$ (D_4^2, No. 19), from systematic absences; $Z = 4$, $D_c = 1.21$ gcm^{-3}; molecular weight calc. 1034.03, found 1031. Mo Kα-radiation, λ taken as 0.7107 Å, $\mu = 7.3$ cm^{-1}. The limits of errors are given in the form of maximum errors.

The intensity measurements were made on a Siemens AED automatic four-circle diffractometer at room temperature. Because of the weak scattering of the crystals available, only 1147 independent reflections were recorded. The maximum value of sin Θ/λ was 0.44, corresponding to a resolution of 0.7 Å. Absorption corrections were not applied, nor were extinction corrections.

The coordinates of the iodine atoms were first determined without ambiguity from a three-dimensional PATTERSON synthesis, sharpened on iodine, corresponding to an R value of 48%. The structure was then completely solved by a combination of FOURIER methods and block-diagonal least-squares calculations. The final refinements gave an R value of 10.1%[2].

Because of the poor resolution of the FOURIER syntheses, the following additional crystallographic evidence was used to determine unambiguously the nature of the atomic species and the positions of the double bonds: 1. local analysis of the variation of the thermal parameters, depending upon the choice of scattering curve for each atom; 2. stereochemical considerations such as valence angles and the approximate coplanarity of certain groups of atoms. In the figure a general projection of compound V is given, which shows that venturicidin A is a non-polyenic macrolide antibiotic.

The sugar moiety is bound to the aglycone through the oxygen atom on C-13, forming a β-glycosidic linkage. As no anomalous dispersion measurements were made, the correct absolute configuration, which is shown in the Figure, was deduced from the known configuration of D-rhamnose[3].

Because of the low resolution, in the FOURIER synthesis, of the side chain of the molecule, due to the large thermal motion as well as to the probable disorder of the final atoms of the chain, strict crystallographic evidence is lacking for the terminal methyl group (C-27). However, the following chemical evidence unambiguously demonstrates its presence.

Ozonolysis of Di-O-acetylventuricidin A. The ozonolysis was carried out in methylene chloride at −70 °C. Destruction of the ozonides with H_2O_2 and HCl in acetic acid, methylation by diazomethane, acetylation and column chromatography on silica gel yielded the following products:

1. 1,4-di-*O*-acetyl-3-*O*-carbamyl-2-deoxy-D-rhamnose, identified by its NMR-spectrum.

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[2] All the calculations were carried out on the Univac 1108 computer of the University, Rome, and were performed with the system of programs developed in Laboratorio di Strutturistica Chimica 'Giordano Giacomello' – CNR. The figure was produced by Johnson's ORTEP program.

[3] B. ISELIN and T. REICHSTEIN, Helv. chim. Acta *27*, 1146 (1944).