exclude the possibility that liver cytochrome *c* may move to other tissues, nor do they exclude the possibility for movement from tissue to tissue of mobilizable smaller molecule precursors.

The "turnover" rate of liver cytochrome *c* is regarded as provisional, since at present we possess no information on the glycine pool in localized areas of the body. Degradation of the labelled chromoproteins, now in progress, will reveal whether the ratio of C^{14} incorporation in hemin and in the protein moiety is different for the cytochrome *c* of different tissues and for hemoglobin. It is also desirable to know whether the metabolism of cytochrome *c* in tissues is dynamic, or "static" as in the case of hemoglobin in mature red cells.

Summary. The participation of glycine in the biosynthesis of cytochrome *c* has been demonstrated. Under our conditions, the incorporation of C^{14} from glycine-2-C^{14} was of relatively very low order in the cytochrome *c* of heart and very high order in liver tissue which had undergone active regeneration. The data support the conclusion that in regenerating liver cytochrome *c* is fabricated *in situ* and not derived from other tissues. A rate of "turnover" for liver cytochrome *c* has been provisionally deduced, which is appreciably higher than the normal rate of hemoglobin "turnover." It is tentatively proposed that chromoprotein biosynthesis is a general property of living, aerobic cells.

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Liver Injury Following Administration of α- and β-Longilobine.* (18545)

F. G. HENDERSON, P. N. HARRIS, AND K. K. CHEN.
From the Lilly Research Laboratories, Eli Lilly and Co., Indianapolis.

The *Senecio* species first attracted attention when they were found to be responsible for the death of cattle and horses in Canada, South Africa, New Zealand, and the United States. Cushny(2) described the effects of 2 alkaloids, senecifolin and senecifolidine, both isolated from *Senecio latifolius,* in dogs, cats and rats. Since that time some 40 alkaloids of *Senecio* have been studied. One of these is longilobine which occurs in *Senecio longilobus,* a plant indigenous to western Texas, and responsible for great cattle losses. Clawson(3) showed *Senecio longilobus* to be one of the more poisonous of the American species of *Senecio.* Horses, sheep and cattle succumbing to a diet of this plant were found to suffer from extensive liver damage. Longilobine was isolated by Manske(4). Adams and Govindachari(5) employing a chromatographic procedure were able to separate longilobine into two components, α- and β-longilobine. Chemical investigation by Warren and his associates(6) proved that β-longilobine is identical in structure with retrorsine.

Previous studies in this laboratory(7-9) showed that Manske's longilobine and retrorsine caused liver damage in white mice follow-

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ing intravenous injection. No pharmacological information is as yet available in regard to the relative activity of the 4 closely related alkaloids. Professor Roger Adams, Department of Chemistry, University of Illinois, Urbana, kindly supplied us with α- and β-longilobine. The object of the present work was to make a direct comparison of these two alkaloids with Manske's longilobine and retrorsine in albino mice.

One per cent solutions of longilobine, α- and β-longilobine were prepared by neutralizing the required amount of each base in an equimolecular quantity of hydrochloric acid, the latter being employed in N/10 solution. Retrorsine was employed as hydrochloride in aqueous solution. The mice came from the same colony. In one experiment, Manske's longilobine and Adams' α- and β-longilobine were injected intravenously on the same day. In another experiment, retrorsine and β-longilobine were injected on the same day, also by vein. Five dose levels expressed by the weight of the base were used with α- and β-longilobine, and four dose levels, with Manske's retrorsine and longilobine. Groups of 5 to 10 mice were injected at each dose level. Following injection the animals were placed in individual cages for observation. All animals that died were studied pathologically as soon as possible to avoid post mortem changes. The experiment was terminated at the end of the fifth day following injection. It was our experience that if they had survived 5 days, they would recover.

The results are summarized in Table I. Those for Adams' β-longilobine from 2 tests were so close that they were combined. By inspection it will be noted that the median lethal dose ± standard error ($LD_{50} \pm$ S.E.) of longilobine matches that of β-longilobine, and the LD_{50} of α-longilobine matches that of retrorsine. When calculated by the combined slope method(10) no statistical difference could be detected among the 4 alkaloids. For example, the apparent difference in LD's between β-longilobine and retrorsine which are chemically identical is not significant at the 5% point.

TABLE I. Acute Toxicity in Mice.

Alkaloid	Dose (as base) mg/kg	No. mice died / No. used	$LD_{50} \pm$ S.E. (as base) mg/kg
Longilobine	62	2/5	76.86 ± 4.84
	70	1/5	
	80	2/5	
	90	4/5	
α-Longilobine	62	1/5	71.52 ± 4.65
	70	1/5	
	80	5/5	
	90	4/5	
	100	5/5	
β-Longilobine	62	0/5	77.20 ± 5.00
	70	2/5	
	80	9/10	
	90	6/10	
	100	10/10	
Retrorsine	56	0/5	71.67 ± 2.93
	63	3/10	
	73	5/10	
	82	7/9	

Of the mice injected, 6 died immediately—2 with longilobine, 2 with α-longilobine, and 2 with β-longilobine. No pathological examinations were made with these animals since there was not enough time for any lesion to develop. One mouse in the β-longilobine group was lost. Sixty animals that died in 7-72 hours were subjected to necropsy—7 on longilobine, 14 on α-longilobine, 24 on β-longilobine, and 15 on retrorsine.

Pathologically, the liver was unquestionably the focal organ of attack. An overwhelming majority of the livers of those mice that died from the 4 alkaloids had a mottled appearance. Many of the lobules were accentuated by central congestion with peripheral pallor. Microscopically, central necrosis with sinusoidal congestion and hemorrhage into cell cords was the predominating picture—5 with longilobine, 14 with α-longilobine, 13 with β-longilobine, and 8 with retrorsine. Infiltration of macrophages into necrotic areas was observed twice with longilobine and once with β-longilobine. In addition midzonal and periportal necrosis of the liver occurred twice with longilobine, and midzonal necrosis, once with retrorsine. One liver in the β-longilobine group showed infectious necrosis which was not attributable to the alkaloid. The livers of 10 mice were not affected by small

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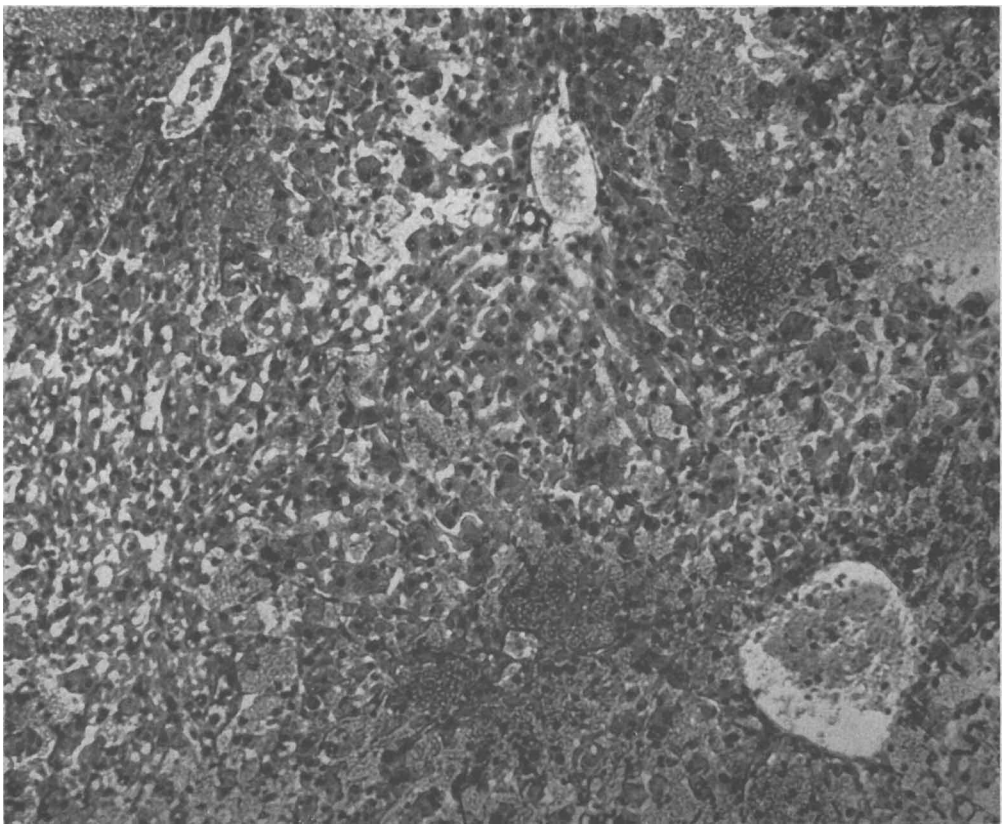

FIG. 1.
Liver damage produced by β-longilobine. This section is from the liver of an albino mouse which died 7 hr after receiving β-longilobine in a dose of 110 mg per kg by vein (Tellyesniczky's fixative, hematoxin-eosin, ×130). Note the central and midzonal necrosis and hemorrhage.

doses of β-longilobine. and those of 6 others, not by retrorsine.

The lungs were occasionally involved. Slight pulmonary oedema was present in 2 mice injected with longilobine, in 4 mice injected with α-longilobine, and in 5 mice injected with β-longilobine. In the last group, hydrothorax occurred in one animal, and broncho-pneumonia in another.

The above data indicate very clearly that all 4 alkaloids, injected intravenously to mice, have the same toxicity and the same type of hepatotoxic action. What difference exists among them elucidated by chemical means is not demonstrable toxicologically and pathologically. This is in contrast with optical isomers of sympathomimetic amines(11) and allo- and normal isomers of cardiac glycosides (12), which are easily distinguishable by pharmacological tests.

Summary. Four closely related alkaloids —longilobine, retrorsine, α- and β-longilobine —have been compared in mice by intravenous injection. There is no significant difference in their toxicity as measured by the median lethal doses. All 4 substances produce liver damage with central necrosis as the predominating lesion.

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