

Table 1. Preparation of ethoxalyl esters

						$\begin{array}{c} \text{COCO}_2\text{C}_2\text{H}_5 \\ \text{RCH} \\ \text{CO}_2\text{C}_2\text{H}_5 \end{array}$
R	Yield %	B.p.		Equivalent weights Calc.	Found	
Ethyl	60	104/4 mm		216.2	217.7	
Isopropyl	20	100/2 mm		230.3	236.5	
<i>n</i> -Propyl	63	99/1.5 mm		230.3	232.4	
Isobutyl	57	102/1.5 mm		244.3	250.2	
<i>n</i> -Butyl	61	116/2 mm		244.3	249.4	

1. Adickes, F., and Andresen, G. *Ann.* **555** (1943) 48.

2. *Organic Syntheses II* (1943) 272.

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## On the Action of Bacterial L-lysine Decarboxylase on Hydroxylysine

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Gale<sup>1</sup> has shown that various bacteria possess an enzyme capable of specifically decarboxylating L-lysine. He also tested the purified enzyme against different lysine derivatives and found it inactive in all cases except when hydroxylysine was the substrate. The hydroxylysine used was an impure preparation (85 % periodate-ammonia,  $(\alpha)_D + 4.7$  in *N* HCl) that was decarboxylated to about 60 per cent although at a slower rate than lysine.

However, Zittle and Eldred<sup>2</sup> have claimed that L-lysine decarboxylase does not decarboxylate hydroxylysine. This question is obviously of importance for the quantitative determination of L-lysine with this enzyme.

Using pure hydroxylysine prepared according to Bergström and Lindstedt<sup>3,4</sup> we have reinvestigated this question. The results plotted in Fig. 1 show that hydroxylysine was decarboxylated to the same extent as lysine, although at a considerably slower rate. It is evident, therefore, that any hydroxylysine present will give an error in the determination of lysine with the usual preparation of bacterial decarboxylase as the carbon dioxide evolved represents the sum of L-lysine and hydroxylysine present.

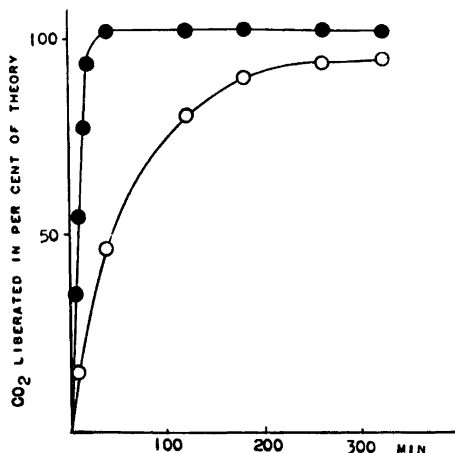


Fig. 1. ○ hydroxylysine. ● lysine. The values are corrected for CO<sub>2</sub> retention in the solution.

We have obtained one mole of carbon-dioxide per mole hydroxylysine on preparations from fish skin. The analytically pure samples from some commercial gelatines give less, possibly due to partial racemization in the manufacturing process. Synthetic hydroxylysine prepared according to Touster<sup>5</sup> was decarboxylated to 25 per cent while a synthetic L-hydroxylysine obtained from Dr. J. Weissiger showed 50 per cent decarboxylation. This work will be reported in detail in a subsequent publication.

*Experimental.* *Bacterium cadaveris* (strain no. 6578 Natural collection of type cultures, London) were grown and acetone dried as described by Zittle and Eldred. 5  $\mu$ M of the amino acid in 0.5 ml water

was shaken at 30° with 2.5 mg bacterial powder suspended in 2.5 ml of 0.2 m phosphate buffer at pH 6.0.

My sincere thanks are due to Dr. Rune Grubb for assistance in culturing the bacteria.

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2. Zittle, Z. D., and Eldred, N. R. *J. Biol. Chem.* **156** (1944) 401.
3. Bergström, S., and Lindstedt, S. *Arch. Biochem.* **26** (1950) 323.
4. Bergström, S., and Lindstedt, S. *Acta Chem. Scand.* **5** (1951) 157.
5. Touster, O. *J. Am. Chem. Soc.* **73** (1951) 491.

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## The Complex Nature of the „Labile Factor” in Chicken Plasma and the Characterization of the Individual Components

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During the study of the coagulation anomaly produced in chicks by vitamin K-deficiency and dicumarol poisoning, it has been demonstrated, in this laboratory, that these two coagulation anomalies are not identical<sup>1-3</sup>.

Dicumarol causes depression of both prothrombin and the  $\alpha$ -factor<sup>2</sup>, while in vitamin K-deficiency low levels of both prothrombin and the  $\delta$ -factor are found<sup>3</sup>. A common property of these three coagulation factors — prothrombin,  $\alpha$ -factor and  $\delta$ -factor — is their adsorbability by  $\text{BaCO}_3$  and  $\text{SrCO}_3$ .

During these studies a rather sensitive method for determination of the labile

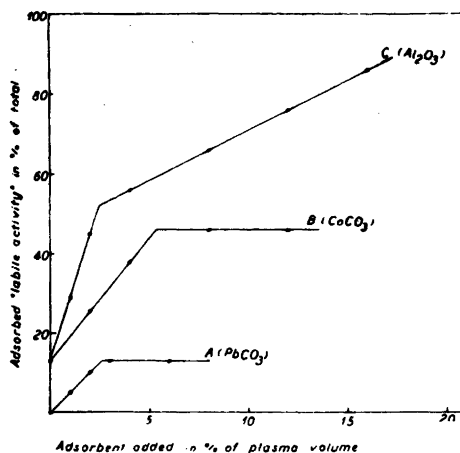


Fig. 1.

Curve A: effect of  $\text{PbCO}_3$  on plasma treated with  $\text{SrCO}_3$ .

Curve B: effect of  $\text{CoCO}_3$  on plasma treated with  $\text{SrCO}_3$  and  $\text{PbCO}_3$ .

Curve C: effect of  $\text{Al}_2\text{O}_3$  on plasma treated with  $\text{SrCO}_3$  and  $\text{PbCO}_3$ .

activity of fresh plasma, viz. ability to restore the prothrombin time of stored oxalated chicken plasma, was developed using stored oxalated plasma as a substrate. It could be shown that adsorption with  $\text{BaCO}_3$  or  $\text{SrCO}_3$  sometimes, but not always, would reduce the labile activity of fresh plasma. The degree of reduction depends on the storage time of the plasma used as substrate and indicates a slow inactivation of the  $\alpha$ -factor and possibly also of the  $\delta$ -factor during storage.

The determination of the labile activity — after total removal of prothrombin,  $\alpha$ -factor and  $\delta$ -factor by  $\text{SrCO}_3$ , has now made it possible to compare the labile activity of different plasmas and to investigate in detail the effect of various adsorbents on the labile activity of fresh plasma.

It is regularly found that the labile activity of different fresh chicken plasmas may show great differences — depending on the diet and the length of time during

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