

TABLE II.

Enzymatic Hydrolysis of β -Chloroethyl Acetate and Acetylcholine.

Same method as described in Fig. 1; same enzyme preparations as in Table I. Final concentration of acetylcholine: 0.0067 M; final concentration of β -chloroethyl acetate; 0.033 M for the first three and 0.016 M for the last preparation. Q_{ACh} resp. $Q_{\beta C1A}$ give the number of microliter of CO₂ produced in 1 hour, calculated from reaction of zero order.

Source of cholinesterase	Type	Q_{ACh}	$Q_{\beta C1A}$
Erythrocytes (human) purified	e	669	249
Snake venom (Naia melanoleuca)	e	540	129
Plasma (human)	s	405	39
Parotid (guinea pig)	s	711	72

split this ester (Table II).

Detailed information about methods and enzyme and substrate preparations will be given in an extensive publication.

Conclusions. The ability to hydrolyze non-choline esters can no longer be used as a criterion to distinguish the two cholinesterase types, since both groups of enzymes are able to split noncholine esters; for example, ethyl chloroacetate. Another noncholine ester, β -chloroethyl acetate, is even preferentially attacked by the e-type ("true" or "specific" ChE).

The use of eserine to decide whether a hydrolysis is catalyzed by a cholinesterase or another esterase is of limited value, because the s-type ("pseudo" or "unspecific" ChE) is inhibited by this substance only in the presence of acetylcholine, but not in that of ethyl chloroacetate. The e-type is inhibited in both cases.

Ethyl chloroacetate plus eserine and β -chloroethyl acetate can be used as new means of distinction of the two groups of cholinesterases.

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Variations in the Vitamin B₁₂ Content of Selected Samples of Pork and Beef Muscle.*

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In a recent paper¹ a fairly quantitative assay procedure for a growth factor in liver preparations was described. This assay procedure involves placing rats on the basal ration for a 2 week depletion period and following the growth response during another 2 week period when the material to be tested

is given. Further work has shown that crystalline vitamin B₁₂ will give a quantitative response in this assay (Fig. 1).

Henderson *et al.*² have reported wide variations in the ability of pork muscle, when fed to female rats as the source of protein, to produce normal lactation. Less than one-third of the young were reared in certain experiments; however, in other experiments the lactation was comparable to that obtained with rations containing beef. Beef-fed rats

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¹ Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, in press.

² Henderson, L. M., Schweigert, B. S., Mozingo, A. K., and Elvehjem, C. A., *J. Nutrition*, 1948, **36**, 479.

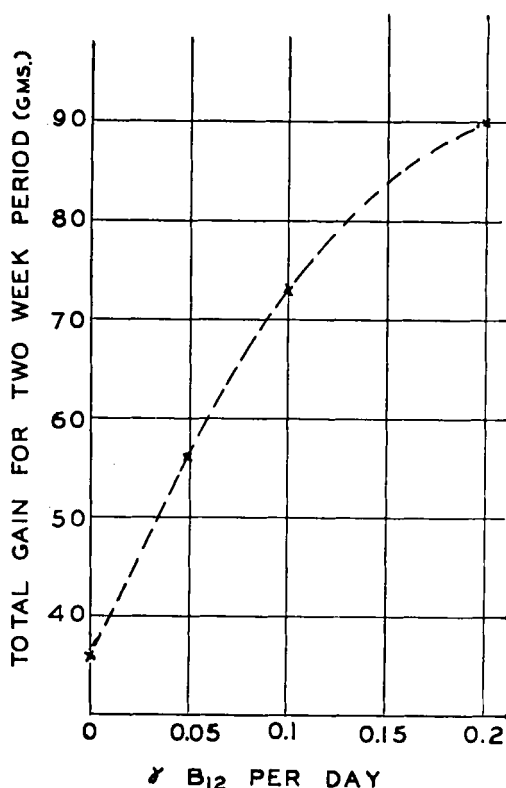


FIG. 1.

Response of rats receiving graded levels of B₁₂.

usually reared 80 to 90% of the young. The wide variations encountered when pork was fed as the source of protein led to the testing of samples of beef and pork by this new assay

method for B₁₂ activity.

A commercial liver preparation, assayed for vitamin B₁₂ content by means of rats using crystalline vitamin B₁₂ as a standard, was used as a reference. One USP antipernicious anemia unit of liver preparation was found to contain approximately one microgram of vitamin B₁₂.

These results show that the beef samples contain twice as much B₁₂ as the normal pork sample while the abnormal pork sample contains only a trace or no B₁₂. The results given for the meat samples are minimum values since they are compared with B₁₂ which was injected intraperitoneally. The wide variation in the B₁₂ content of pork samples is significant in that it may offer an explanation for the variations in the ability of pork samples to produce normal lactation in female rats reported by Henderson and co-workers.²

The variable results with pork are not surprising since swine, being monogastric animals, may not be supplied with ample quantities of B₁₂ and possibly other factors which may be produced by the microorganisms in the rumen of cattle.

We are indebted to Merck and Co., Inc., Rahway, N. J., for crystalline vitamins including B₁₂, and to Dr. B. L. Hutchings of the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., for synthetic folic acid.

Daily supplement	Total avg gain (g) two wk	Min. B ₁₂ (γ) per 100 g of sample
None	36	—
5 g beef, round steak (Sample 1)	74	2
5 g beef, round steak (Sample 2)	74	2
5 g pork, shoulder (normal)*	56	1
5 g pork, shoulder (abnormal)†	39	Trace
0.1 USP. unit Reticulogen (Lilly)‡	74	2000§

* From sow that lactated normally.

† From sow that showed abnormal lactation (young died after birth).

‡ Injected intraperitoneally.

§ Contains approximately 1 γ B₁₂ per USP unit.