binding between FFA and albumin because of the liver damage induced.

In accordance with previous studies[1-3,12] the plasma neutral lipids were decreased in the ethionine treated rats with a concurrent increase in liver lipids (Table). Plasma free fatty acids were, on the other hand, increased compared to control animals (Table). The increased FFA level may be related to the enhanced electrophoretic mobility of the α-LP (Figure) observed in the ethionine treated rats[13,14].

Since pre-α-LP might hold a substantial part of the plasma lipid and is affected by hepatic as well as nutritional and hormonal factors[15,4], it may play an important role in lipid metabolism and in certain hyperlipoproteinemias.

Zusammenfassung. Nach Ethionin-Behandlung wird im Rattenplasma eine Reduktion des Pre-α-Lipoproteins festgestellt.

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[12] N. M. Papadopoulos, C. M. Bloor and J. C. Standefer, J. appl. Physiol. *26*, 760 (1961).
[13] S. Koga, D. L. Horwitz and A. M. Scanu, J. Lipid Res. *10*, 577 (1969).
[14] E. Schafrir, J. clin. Invest. *37*, 1775 (1958).
[15] J. Dyerberg and N. Hjørne, Clin. chim. Acta *28*, 203 (1970).

The Presence of Pectin Methylesterase in Cacao Pulp

An essential first stage in the preparation of cacao beans for the market is the fermentation or curing process. During the fermentation of the pulp the sugars are converted into alcohol. Simultaneously, the cells of the pulp are broken down. The solutions which arise from the fermentation are called 'sweatings'. Presumably, pectic enzymes are involved in the latter process. Hydrolysis of the pulp as observed during the curing process also occurs with ripe fruit before it is removed from the pod. Complete pectin hydrolysis by pectinase must be preceded by deesterification by pectin methylesterase[1]. Therefore, it was decided to test for the presence of pectin methylesterase and estimate its activity in the pulp at various stages of ripeness. Pectin methylesterase activity increased as the fruit ripened, suggesting that the enzyme activity is increased at a stage when the pulp is to be removed and the cacao seeds exposed.

Experimental. The fruit of cacao (Amazon) was extracted with 0.5 *N* sodium acetate pH 7.8 (200 ml per pod) overnight in the cold, removing the pulp from the beans by cutting with a sharp scapula. The pulp was removed without damaging the beans. The extract obtained by decanting the mixture and leaving the beans behind, was centrifuged at 12,100 × *g* in a Sorvall refrigerated centrifuge. The latter supernatant was the crude enzyme extract. Insoluble pulp was discarded. Activity was determined by maintaining the pH constant by titrating with 0.02 *N* NaOH using 0.5% pectin (10.86% methoxyl content) in 0.1 *M* NaCl as the substrate[2]. A unit of pectin methylesterase activity was defined as that removing 1 μmole of methoxyl groups in 10 min at 25 °C.

Pectin methylesterase was partially purified by fractionating with 50% ammonium sulfate. The activity appeared in the precipitate fraction, which was redissolved in 0.5 *M* sodium acetate and the insoluble material removed by centrifuging at 12,100 × *g*. Protein content was determined by the standard Kjehldahl method. The enzyme was purified about 55-fold (specific activity of the crude extract was 6 μmoles methoxyl/mg protein and of the ammonium sulfate fraction 330 μmoles methoxyl/mg protein).

Results and discussion. In young cacao pulp (70–120 days) no pectin methylesterase activity was detected. Activity appeared in the ripe fruit and increased with degree of ripeness (as estimated by color of the pod), Table. At pH 5.0 no significant activity was observed; with increasing pH the activity was elevated. 1.2, 1.6, 3.3, 1.5 and 2.6 μmoles methoxyl/mg protein were released at pH 6.0, 6.5, 7.0, 7.5, and 8.0 respectively. Activity was greatest at pH 7.0, but it is hazardous to conclude the latter is an optimum pH, as Kertesz[3] has indicated. The apparent increase at pH 8.0 may be due to substrate instability[3]. The presence of pectin methylesterase in the pulp and its increase with degree of ripeness suggests that it is important in the initial stages of removal of the pulp from the bean. Pectin methylesterase activity has been found to increase with ripeness in banana[4] and tomato[5]. It appears that the enzyme is involved in the ripening process in cacao. In the case of the cacao, its activity may be important in determining the quality of the bean. The pectic substance content is, undoubtly, of importance in determining the texture of cocao made from the beans. Pectin methylesterase can be employed in the processing of orange juice, tomato aspic, salads and puddings. Thus, the enzyme is potentially and economically important by-product of the cacao industry in Ghana.

Zusammenfassung. Die Pektin-Methylesterase wurde aus Kakaofruchtfleisch (Amazon) isoliert und teilweise gereinigt, wobei die Fermentaktivität sich mit zunehmender Reife der Kakaobohne erhöhte.

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Pectin methylesterase activity in developing cacao fruit

Stage	μmoles Methoxyl/mg protein
70 days	*
90 days	*
120 days	*
Ripe (175–185 days)	
I (greenish yellow)	3.3
II (yellow)	4.0
III (redish orange)	13.0

* No detectable activity when tested under standard assay conditions.

[1] C. E. Hobson, Biochem. J. *86*, 358 (1963).
[2] *Worthington Enzymes* (Worthing Biochemical Corporation, Freehold New Jersey 1969).
[3] Z. I. Kertesz, *The Pectic Substances*, (1951), p. 369.
[4] H. O. Hultin and A. S. Levine, J. Food Sci. *30*, 917 (1965).
[5] G. E. Hobson, J. Sci. Food Agric. *14*, 550 (1963).
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