Table II. Responses of honey bees to either 1% nitrobenzene vs. Nujol or 1% nitrobenzene-$d_5$ vs. Nujol when conditioned to 1% nitrobenzene

| Time test began (h) | Time for 50 bees to land (min) | Bees responding to nitrobenzene (%) | Bees responding to nitrobenzene-$d_5$ (%) |
|---|---|---|---|
| 09.52 | 5.5 | 68 | |
| 10.26 | 5 | | 56 |
| 11.15 | 6 | 70 | |
| 11.52 | 5.5 | | 64 |
| 13.24 | 6.5 | 62 | |
| 14.10 | 4 | | 62 |
| 14.51 | 4 | 48 | |
| 15.34 | 5.5 | | 66 |
| Mean | | 62 | 62 |

Table III. Responses of honey bees to 1% nitrobenzene vs. 1% nitrobenzene-$d_5$ when conditioned to 1% nitrobenzene

| Time test began (h) | Time for 50 bees to land (min) | Bees responding to nitrobenzene (%) | Bees responding to nitrobenzene-$d_5$ (%) |
|---|---|---|---|
| 09.04 | 4.5 | 54 | 46 |
| 09.55 | 4.5 | 52 | 48 |
| 10.50 | 5 | 46 | 54 |
| 11.34 | 5 | 42 | 58 |
| 13.02 | 3.5 | 54 | 46 |
| 13.41 | 4.5 | 54 | 46 |
| 14.30 | 5 | 56 | 44 |
| 15.11 | 8.5 | 46 | 54 |
| Mean | | 51 | 49 |

peaks assignable to $-NO_2$ were shifted by interactions. Thus, aside from some similarity in the peaks assigned to $-NO_2$ stretching near 1525 and 1350 $cm^{-1}$, the spectra of nitrobenzene and nitrobenzene-$d_5$ have no peaks in common.

A bioassay that utilizes honey bees, *Apis mellifera* L.[7], was employed. Bees were conditioned for 6 h to associate the odor of 1% chemical in mineral oil with the availability of 30% sucrose solution. Test scents were put onto filter paper in each of 6 beakers 5 cm below the aroma ports of a test arena that rotated at 1/3 rpm. A metal screen was placed over each port and changed after a bee had landed to reduce the probability that bee odor influenced the selection of an aroma port by the bees[8]. The results of at least 4 tests (50 visits per test) were used to calculate a pooled chi square value which determined the statistical significance of our results.

*Results.* When bees were allowed to choose between 1% nitrobenzene and 1% benzaldehyde, both odors described[3] as 'bitter almond', the results (Table I) showed conclusively that bees distinguished between these 2 similar compounds with 73.5% of the bees responding to benzaldehyde ($P < 0.01$).

When bees were conditioned to nitrobenzene and were allowed to choose between nitrobenzene vs. control or nitrobenzene-$d_5$ vs. control, the higher numbers of bees visiting scented beakers (Table II) were highly significant ($P < 0.01$). Although nitrobenzene vapor can be poisonous to some animals and benzaldehyde is sometimes used as a bee repellent, the conditioned bees did use the aromas to find sugar solution. The minutes elapsed in each test before 50 bees landed did not indicate any delay in response to the deuterated compound as compared with the undeuterated. Thus, bees conditioned to nitrobenzene responded identically to both isotopic analogs.

In a more critical comparison the bees chose between 3 beakers that contained 1% nitrobenzene and 3 that contained 1% nitrobenzene-$d_5$. The results further support the contention that conditioned bees did not distinguish nitrobenzene from nitrobenzene-$d_5$ (Table III).

*Discussion.* Present indications are that the receptor cells for olfaction in honey bees, like those in melon flies and ants, do not allow the insect to distinguish isotopic analogs of certain odorous chemicals. Such findings augment other results based on odor differences in enantomers[9] to support a concept that electronic structure and force fields are involved in odor discrimination.

*Zusammenfassung.* Honigbienen, *Apis mellifera* L., wurden dressiert, Zuckersirup an Stellen zu sammeln die mit Nitrobenzol markiert waren. Wenn Nitrobenzol oder Nitrobenzol-$d_5$ zur Auswahl standen, konnten die Bienen nicht zwischen diesen Analogen unterscheiden. Dieses Ergebnis stimmt mit der Überlegung überein, dass die Chemorezeptormechanismen mit Elektronenmustern oder -effekten zusammenhängen und nicht auf Oszillations- und Rotationsfrequenz, Dipolmoment oder anderen Masseneffekten an Molekülen beruhen.

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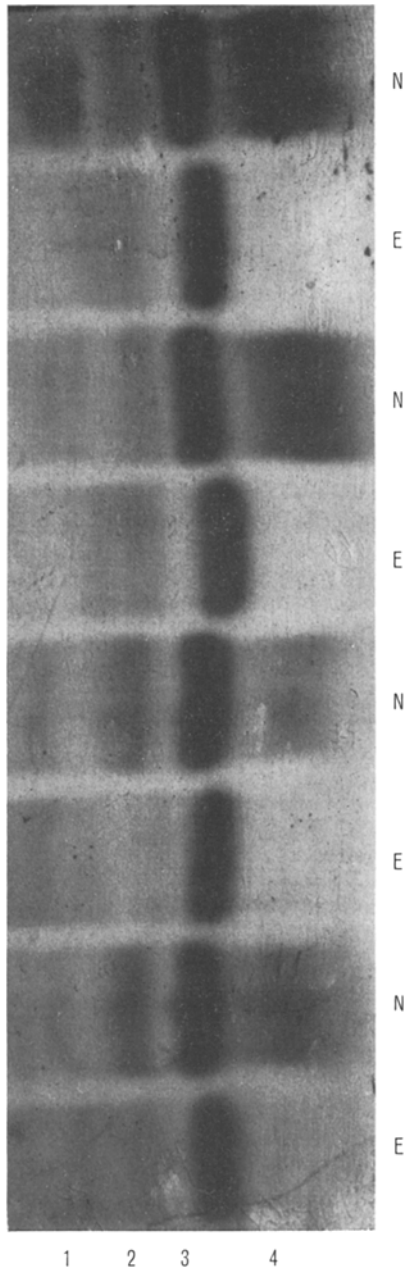
## Plasma Pre-α-lipoproteins in Ethionine Induced Fatty Liver in Rats

It is well known that ethionine interferes with the synthesis of β-, pre-β- and α-lipoprotein in the liver[1–3]. No effect of ethionine on pre-α-lipoprotein, however, has been reported. In the present communication a very specific effect on pre-α-lipoprotein synthesis in the liver by ethionine treatment is reported.

Female rats of Wistar strain, having a mean weight of 200 g, were used. All rats were fed on a diet rich in carbohydrates for 24 h together with drinking water enriched with 20% D-glucose. The animals were then fasted for 24 h and given ethionine injections i.p., 4 injections of 50 mg each[2]. The fasting control rats received 0.9% NaCl




Lipoprotein electropherograms of rat plasma. N, normal fasting control animals; E, rats with ethionine induced fatty liver; 1, $\beta$-lipoprotein; 2, Pre-$\beta$-lipoprotein; 3, $\alpha$-lipoprotein; 4, Pre-$\alpha$-lipoprotein.

injections only. After the infusions, the animals were given only drinking water without glucose. 24 h later, the rats were exsanguinated by heart puncture (under ether narcosis).

The liver was removed for macroscopical and microscopial investigations. A fulminant fatty liver was developed in the ethionine treated rats, evidenced macroscopically by an increase in liver weight and a yellow appearance and verified by microscopy after Sudan staining.

Lipoprotein electrophoresis was performed on 1% agarose gel as recently reported[4]. Analyses of plasma triglycerides were performed according to LAURELL[5] and plasmacholesterol was determined according to CARR and DREKTER[6]. The homogenization of liver tissue and determination of liver lipids were carried out according to FOLCH et al.[7]. Plasma free fatty acids were determined by a micro method of DOLE and MEINERTZ[8].

In control animals the pre-$\alpha$-LP showed a heavy uptake of fat stain (Figure) indicating that pre-$\alpha$-LP is an important transport vehicle for lipids in rat plasma. Ethionine treatment produced a specific lowering of the pre-$\alpha$-LP level in plasma (Figure). The reason for this is still unclear and has to be further investigated.

It has been previously suggested that pre-$\alpha$-LP is a genuine lipoprotein carrying mainly lysolecithin and small amounts of neutral lipids, presumably triglycerides[9]. Others, however, reported that the pre-$\alpha$-LP is identical with the albumin-FFA complex[10,11]. The specific reduction in pre-$\alpha$-LP level (Figure) by ethionine treatment demonstrated in this study, cannot be related to a decrease in the albumin-FFA complex, since the FFA level was found to be increased in the same animals. This observation agrees with the previous report[9] that the pre-$\alpha$-LP (density $> 1.21$) is a genuine lipoprotein and not identical with the albumin FFA complex. Since FFA are bound to albumin in the peripheral adipose tissue, the reduction in the pre-$\alpha$-LP cannot be related to reduced

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Effect of ethionine on plasma and liver lipids

| Treatment | Cholesterol | | Triglycerides | | Free fatty acids | Total protein |
|---|---|---|---|---|---|---|
| | Plasma (mg/100 ml) | Liver (mg/tissue)[b] | Plasma (mg/100 ml) | Liver (mg/g tissue)[b] | Plasma ($\mu$Equiv./l) | Plasma (g/100 ml) |
| None Controls | 47.0 | 4.0 | 89.0 | 26.0 | $633 \pm 41$[a] | 5.2 |
| Ethionine | 14.0 | 5.7 | 32.0 | 74.0 | $741 \pm 124$[a] | 5.0 |

[a] One standard deviation. [b] Wet weight.

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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## 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.

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