standards. Mass spectra data were particularly useful to identify the peaks corresponding to the $C_{18:3}$ and $C_{20:1}$ unresolved in our gas chromatographic conditions, and the minor component $C_{22:1}$. In the first case the MS of the unresolved chromatographic peak showed the presence of both the parent peaks of $C_{18:3}$ and $C_{20:1}$ at *m/e* 292, and 324 respectively. The peaks at *m/e* 292 (M-32), *m/e* 236 (M-56) and 223 (M-69) for $C_{18:3}$ and at *m/e* 292 (M-32), *m/e* 250 (M-74) and *m/e* 208 (M-116) for $C_{20:1}$ were also observed. $C_{22:1}$ was identified by the parent peak occurring at *m/e* 352 and the characteristic peaks occurring at *m/e* 320 (M-32), *m/e* 278 (M-74) and *m/e* 236 (M-116). Because the mass spectra of positional isomers, with double bond at position 6:7 or higher up in the chain are practically indistinguishable from those of methyl oleate, a comparison with GLC retention time with a pure standard of $C_{22:1}$ was also performed to confirm our findings.

Figures 2a and b show the isocitrate lyase and the malate synthase levels respectively during the observation period. The key enzymes of the glyoxylate cycle increase together and reach their maximum at about the 11th day of germination. These data, compared with the results shown in Table I and in Figure 1, demonstrate that the increase in enzyme levels corresponds to the days in which the lipid catabolism is more evident.

To conclude, we demonstrated the presence and the operativity of the glyoxylate cycle in *Lupinus* seeds, and we confirmed the close correlation between this metabolic pathway and triglyceride catabolism.

Summary. The levels of total lipids and triglycerides were determined during germination of *Lupinus* seeds. The presence and operativity of the two key enzymes of the glyoxylate cycle, isocitrate lyase and malate synthase, were made clearly evident and correlated to lipid metabolism. An oil, relatively free of erucic acid, was obtained from the seeds at 0 germination time.

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The Absence of an Inhibitory Effect of Metyrapone (2-methyl-1,2-di (3-pyridyl) propan-1-one) on Hepatic Microsomal Hydroxylation in Scurvy

There is evidence that the ability of the liver microsomal system to hydroxylate certain compounds is reduced in scurvy[1–3].

DEGKWITZ and STAUDINGER[3] have related the impaired hydroxylation of acetanilide in scurvy to a reduction in the cytochrome P-450 concentration. They pointed out that this would not be inconsistent with an influence of asorbic acid on the biosynthesis of haem and in support of this they point out that cytochrome b_5 too decreases in the microsomes of guinea-pigs deprived of ascorbic acid. FIELDING[4], in a similar type of experiment, also found a scurvy-induced reduction in the microsomal b_5 but found no evidence of a corresponding fall in the cytochrome P-450.

An alternative, or complementary, possibility is that ascorbic acid influences the binding of the enzyme to the substrate. Inhibitors such as Metyrapone act by interference with this binding. An experiment was therefore designed to examine the effect of Metyrapone on the microsomal acetanilide-hydroxylating system from normal and scorbutic guinea-pigs.

Methods. 12 male Dunkin-Hartley guinea-pigs were given a semisynthetic scorbutogenic diet[5]. 6 received a daily supplement of 5 mg ascorbic acid/100 g body-weight and 6 received no supplement. After 12 days the animals were killed by decapitation and exsanguination. Ascorbic acid concentrations were determined in 2 representative tissues, the spleen and adrenals[6]. Liver microsomes were extracted in 3 volumes of ice-cold 1.10% potassium chloride solution followed by homogenization in a Potter-Elvehjem type homogenizer and centrifugation at 9000 *g* at 0–4 °C for 20 min. The supernatant was centrifuged at 38,000 *g* for 60 min (MSE High Speed 18 centrifuge) and the microsomal pellet washed and finally re-suspended in ice-cold 0.2M Sörenson phosphate buffer (pH 7.4) to give a microsomal protein concentration of ca. 10 mg/ml. Liver microsomal protein was measured using the Folin-Ciocaltau reagent[7] and N-acetyl-*p*-aminophenol, the *para*-hydroxylated metabolite of acetanilide, by a modification of the method used by KRISCH and STAUDINGER[8]; no interference by the 'X-product' of KRATZ and STAUDINGER[9] was found.

The hydroxylation incubation mixture contained 2 μmoles nicotinamide adenine dinucleotide, 2 μmoles nicotinamide adenine dinucleotide phosphate, 2.5 μmoles magnesium sulphate, 4 μmoles glucose-6-phosphate, 20 μmoles nicotinamide, 0.2 ml 9000 *g* supernatant, 1–2 mg microsomal protein and the appropriate substrate, in a volume of 1.2 ml. All solutions were in 0.2 phosphate buffer, pH 7.4. Incubation was at 37 °C and the reaction was stopped by the addition of 0.1 ml 20% trichloroacetic acid.

Results and comments. The concentrations of ascorbic acid in the spleen and adrenals respectively were 1.7 ± 0.4 and 3.7 ± 0.7 (mg/100 g fresh tissue) for the scorbutic animals and 11.9 ± 1.2 and 41.7 ± 6.5 for the control animals (mean values with standard errors). The influence of different Metyrapone concentrations on the

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Influence of Metyrapone on the hydroxylation of acetanilide by liver microsomes from guinea-pigs of different ascorbic acid status (μg of product formed)

Metyrapone concentration	0	10^{-6} M	10^{-4} M	10^{-3} M	10^{-2} M
Scorbutic group	17.8	22.0 (+21%)	25.5 (+36%)	29.0 (+51%)	24.5 (+32%)
Controls (5 mg ascorbic acid/100 g body-weight daily)	23.5	30.5 (+31%)	26.5 (+13%)	18.0 (−23%)	16.0 (−32%)

Figures in brackets are the percentage deviations from the non-Metyrapone value.

acetanilide hydroxylation system is summarized in the Table. Scurvy induced a 40% reduction in the hydroxylation system a finding in keeping with earlier observations[3]. No decrease in microsomal protein was found and the addition of ascorbic acid or isomers of ascorbic acid to liver microsomes did not influence the rate of hydroxylation. The 'biphasic' effect of Metyrapone, described by Leibman[10] for 'normal' rat liver microsomal fractions was present. However, scorbutic microsomal hydroxylation was stimulated by Metyrapone at all concentrations used; there was no inhibition at the higher concentration as in the case of microsomes from control animals.

This stimulation by Metyrapone of microsomal acetanilide hydroxylation in scorbutic animals could well indicate a scurvy-induced change in the lipoprotein environment of the membrane-based P-450 enabling a faster transfer of reducing equivalents. A change in liver microsomal membrane conformation in scurvy has also been indicated by a 100-fold increase in the fluorescence emission of the probe 1-anilino-8-naphthalenesulphonate[4].

Summary. Microsomes from livers of scorbutic guinea-pigs showed a reduced rate of acetanilide hydroxylation. The response of 'scorbutic' liver microsomes to the inhibitor Metyrapone (2-methyl-1, 2 di (3-pyridyl)propan-1-one) was different from that of liver microsomes from non-scorbutic guinea-pigs.

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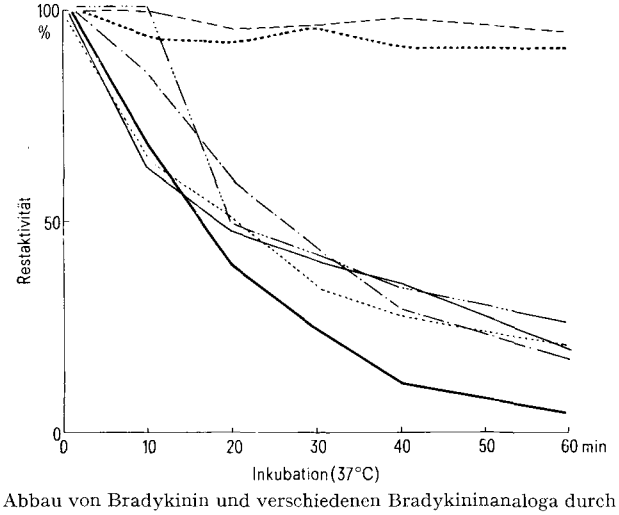
Stabilität verschiedener Bradykininanaloga gegen Kininase II[1]

Stability of some Bradykinin Analogues Against Kininase II[1]

Für Hormon-Rezeptor-Bindungsstudien mit dem Nonapeptid Bradykinin an Membranfraktionen aus Rattenuterus bzw. Rattenduodenum ist es erforderlich, den raschen enzymatischen Abbau dieses Peptidhormons zu verhindern. Das kann mit Enzyminhibitoren oder durch den Einsatz abbaustabiler Bradykininanaloga erreicht werden. So unterdrückt z.B. der Einsatz von *ortho*-Phenanthrolin die enzymatische Inaktivierung des Hormons durch die Mikrosomen- und Membranfraktionen[2]. Es muss jedoch damit gerechnet werden, dass die Anwesenheit von *ortho*-Phenanthrolin die Hormon-Rezeptor-Bindungsuntersuchungen verfälscht. Wir waren deshalb bemüht, kininasestabile Bradykininanaloga mit hoher biologischer Aktivität aufzufinden.



Abbau von Bradykinin und verschiedenen Bradykininanaloga durch die Membranfraktion aus Rattenuterus (75 μg Protein Uterusmembranen pro Inkubationsansatz). ——— Bradykinin; ..... [8-erythro-Phenylserin]-Bradykinin; ooooooo [8-threo-Phenylserin]-Bradykinin; – – – – [8-erythro-α-Amino-β-phenylbuttersäure]-Bradykinin; ····· [8-Cyclohexylalanin]-Bradykinin; –·–·–·– [6-Glycin, 8-Tyrosin]-Bradykinin; –·–·–·–·– [9-Homoarginin-Bradykinin.

Wie wir in früheren Arbeiten durch Einsatz von verschiedenen Kininasehemmstoffen zeigen konnten, enthalten Mikrosomen- und Membranfraktion aus Rattenuterus Kininase II[2]. Dieses strukturgebundene proteolytische Enzym spaltet von dem Nonapeptid Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg das C-terminale Dipeptid ab[3]. Wir haben deshalb eine Reihe von Bradykininanaloga mit Variationen in den Positionen 8 und 9 auf ihren Abbau durch die Membranfraktion aus Rattenuterus und die Mikrosomenfraktion aus Rattenduodenum untersucht.

Methode. Die Synthesen und die biologischen Aktivitäten der als Substrat eingesetzten Bradykininanaloga

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