shifted to either higher or lower values. As shown in the Figure, the approximate pH optimum of aldolase is 8.5; aldolase-EMA 9.0, aldolase-AEC 6.5 and aldolase-PAB 6.0. For the aldolase system, attachment to polymers bearing positive charges, such as AEC, shifts the pH optimum to the acidic side. Thus, when GAPD is attached to AEC, the pH optimum is shifted from 9.0 for GAPD to about 7.0 for GAPD-AEC. When FDPase is attached to AEC, the pH optimum is shifted from about 9.3 to 8.5. It can be concluded that polymers definitely exert vicinal effects on the pH around the catalytic site of the enzymes.

The apparent K_m for substrates can be affected as shown in Table II. When the substrates are negatively charged, polymers bearing negative charges increase the apparent K_m, as in the case of aldolase-EMA. Polymers bearing positive charges decrease the apparent K_m, as in the case of aldolase-PAB and GAPD-AEC.

It is possible to link sequential reactions by appropriate column techniques. Two columns in series containing aldolase-AEC and GAPD-AEC were packed to demonstrate the principle of sequential synthetic reactions. Into the first column, containing aldolase-AEC, was percolated a solution of fructose-1,6-diphosphate and nicotinamide adenine dinucleotide (NAD). The effluent solution contained dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (GAP) and unchanged NAD. This mixture was passed through a second column packed with GAPD-AEC while sodium arsenate was added. GAPD catalyzed oxidation of GAP to 3-phosphoglyceric acid in the presence of NAD and sodium arsenate, and the NAD was reduced to NADH. The effluent from the second column flowed into a solution of methylene blue and diaphorase which were used as specific indicators for NADH. The decolorization of methylene blue proved the presence of NADH which could only have been formed by the projected two-step sequential enzyme-catalyzed synthesis.

Table II. Apparent K_m values for different enzyme-polymer adducts

Enzyme	Substrate	K_m values (mM)
Aldolase	Fructose-1,6-diphosphate	0.04
Aldolase-PAB	Fructose-1,6-diphosphate	0.006
Aldolase-EMA	Fructose-1,6-diphosphate	3.6
GAPD	Glyceraldehyde-3-phosphate	6.4
GAPD-AEC	Glyceraldehyde-3-phosphate	0.66

Résumé. On a preparé des enzymes polymères dérivés de l'aldolase, de l'aldéhyde glycérique-3-phosphate déhydrogénase et de la fructose-1, 6-diphosphatase. On peut, semble-t-il, tirer parti de la technique de liaison transversale par l'aldéhyde glutarique pour augmenter la teneur en protéine et pour obtenir des enzymes polymères adducteurs. Les enzymes traités sont plus stables que les enzymes intacts. La direction des changements du pH optimal et du K_m apparent change selon les polymères auxquels on lie les enzymes.

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Presence of Lipid Bodies in Rice Leaves and their Discolouration During Pathogenesis

Lipids are considered as the third important class of plant constituents after proteins and carbohydrates, and are widely distributed in plant parts. In vegetative parts they are usually in the form of small droplets or globules dispersed through the cytoplasm of the cell[1]. Although the lipid content of the grains of rice (*Oryza sativa* L.) have been investigated[2,3], neither pure anatomical investigations nor pathological studies[4–17] have previously resulted in reports of the presence of these globules in rice leaf tissue.

While observing free-hand sections of rice leaves for anatomical changes during pathogenesis, we found abundant lipid globules in cells throughout the chlorophyll-containing parenchyma tissue of several varieties (Figure). These spherical globules averaged 3.3 µm (range 1.6–4.8 µm). Both healthy and diseased cells contained the lipid globules. Usually 2 or more globules were visible per cell. In healthy tissue the globules appear colourless or light green to yellowish green, presumably because of their location close to chloroplasts. In diseased tissue, the globules become reddish brown to dark brown depending on the nature and stage of disease, and distance from the site of infection. Discoloration of lipid bodies is an early primary symptom of leaf spots resulting from

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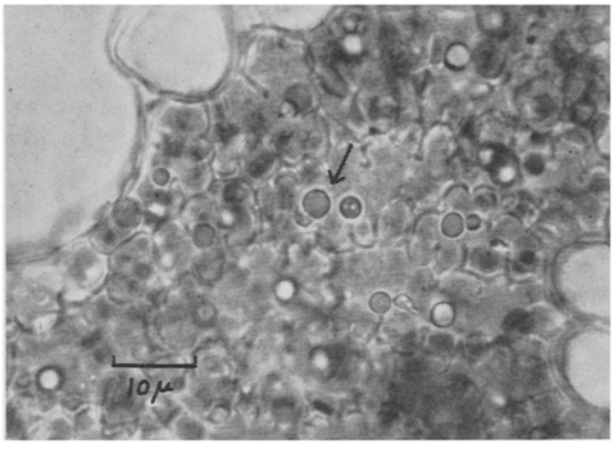
fungi (*Pyricularia oryzae* Cav., *Helminthosporium oryzae* Breda de Haan, *Cercospora oryzae* Miyake), of bacterial leaf streak resulting from *Xanthomonas translucens* f. sp. *oryzicola* (FANG et al.) Bradbury, and of leaf yellowing resulting from virus infection (tungro). With bacterial leaf streak, the conspicuous reddening of the first lesions appears to be caused by the reddening of the abundant lipid droplets.

To identify the globules, we stained hand sections of fresh, healthy rice leaves obtained from 1-month-old plants of variety Peta according to the method of JENSEN[18]. Sudan III stained the globules reddish orange, while Sudan black stained them blue-black, indicating their lipoid nature. The globules stained blue with Nile blue, indicating that they are acidic and either phospholipids or free fatty acids. The globules stained blue-black with acid haematin in hand sections of leaf tissue fixed in Lewitsky's fluid for 18 h, and yellow with orange G-aniline mixture. These 2 reactions show that the globules are phospholipids.



Photomicrograph showing lipid globules in chlorophyll-containing parenchyma cells of rice leaf × 1000.

Because they are abundant in photosynthesizing cells and because they have not been studied in rice leaves, these lipid globules may be of interest physiologically in relation to energy fixation, storage, and metabolism. In addition, their ready discoloration in early stages of disease development may be useful in studies of host-parasite interaction.

Résumé. Pour la première fois, d'abondants globules lipidiques de forme sphérique et d'un diamètre moyen de 3,3 µm ont été observées dans les parenchymes du riz *Oryza sativa* L. Différents colorants chimiques employés en histologie ont permis d'identifier la nature phospholipidique de ces globules lipidiques.

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Formation of N-Glucuronide of Desmethylimipramine in the Dog

Some 20 metabolites are known to be formed from imipramine in humans, rats and guinea-pigs[1-4]. The drug and its secondary amine metabolite, desmethylimipramine, are metabolized mainly along the pathways of aromatic hydroxylation and subsequent *O*-glucuronide formation. When all the unconjugated metabolites are removed from urine or bile by exhaustive extraction, the remaining aqueous phase after hydrolysis yields the phenolic metabolites as aglycones. Although the formation of unidentified glucuronide[2] and non-glucuronide conjugates[3] has been reported, we have obtained what we believe to be the first evidence for N-glucuronide formation as a step in the biotransformation of imipramine and desmethylimipramine by dogs.

Two female boxer dogs (22 and 20 kg) with cannulated urethrae and bile ducts were used. Dog A was given imipramine (4.55 mg/kg p.o.), dog B desmethylimipramine (10.0 mg/kg p.o.). Urine and bile were collected under sterile conditions in 1-h fractions and frozen until use. Extraction, thin layer chromatography, hydrolysis and quantitation were carried out as described previously[1]. The bile and urine samples were first extracted until no unconjugated metabolites could be detected in the last extract. Hydrolysis of the conjugates in the aqueous phase was achieved with Glusulase Boehringer (β-glucuronidase + arylsulfatase), 24 h at 37 °C and pH 5.4. In additional experiments, the two enzymes (Boehringer) were used separately. Non-enzymatic hydrolysis was carried out under appropriate pH and temperature conditions. No spontaneous hydrolysis could be observed during storage of bile and urine at –20 °C over several weeks. The extraction procedures before and after hydrolysis were identical.

Bile of dogs given imipramine (IP) or desmethylimipramine (DMI), after the complete removal of unconjugated material and subsequent hydrolysis, showed not only the expected phenolic metabolites, i.e. aglycones of *O*-glucuronides, but also the basic compounds DMI and desdimethylimipramine (DDMI). As shown in Table I, the amounts of DMI and DDMI released by hydrolysis are for the most part far superior to the corresponding unconjugated metabolites. Furthermore, in the bile of the DMI-treated dog, the amounts of phenolic and basic conjugate are comparable. The identity of the 2-OH-DMI, DMI and DDMI released by hydrolysis was proved by TLC, utilizing 5 different solvent systems. In all cases the Rf-values and colours of the spots on sprayed plates (diazo reagent) were in agreement with those of the authentic compounds as listed in Table II. Finally, since N-glucuronides are known to be more acid-labile than *O*-glucuronides, extracted bile samples of dog B containing equal amounts of both types of glucuronides were hydro-

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