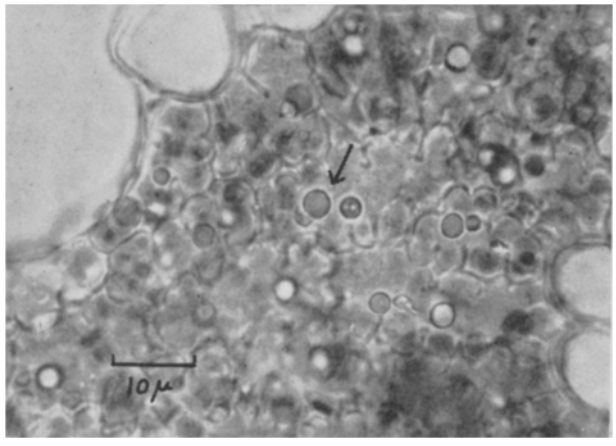
fungi (*Pyricularia oryzae* Cav., *Helminthosporium oryzae* Breda de Haan, *Cercospora oryzae* Miyake), of bacterial leaf streak resulting from *Xanthomonas translucens* f. sp. *oryzicola* (FANG et al.) Bradbury, and of leaf yellowing resulting from virus infection (tungro). With bacterial leaf streak, 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.

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.



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

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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Table I. Metabolite excretion of bile duct cannulated dogs given imipramine or desmethylimipramine

	Dog A given IP[a] Bile			Dog B, DMI[b] Bile	Urine
	µg/4 h	µg/ml 0–1 h	µg/ml 3–4 hr	(µg/4 h)	µg/4 h
Imipramine (IP)	43			–	–
Desmethylimipramine (DMI)	17			trace	trace
Desdimethylimipramine (DDMI)	0			trace	5
IP-N-oxide	200			–	–
2-OH-IP	39			–	–
2-OH-DMI	17			3	270
2-OH-IP-glucuronide	18,900	58	1000	–	–
2-OH-DMI-glucuronide	1,400	21	60	650	750
DMI-conjugate	740	38	5	600	35
DDMI-conjugate	70			trace	15

[a] 100 mg IP p.o. = 4.55 mg/kg. [b] 200 mg DMI p.o. = 10.0 mg/kg.

lyzed under various conditions. As shown in Table III, the conditions required to hydrolyze the DMI conjugate are profoundly different from those for the *O*-glucuronide and are likely to be characteristic for an N-glucuronide.

The results disclose a new metabolite of IP and DMI. The metabolite must be a conjugate since it appears only after various modes of hydrolysis of bile and urine from which unconjugated material has been removed. Its response towards different hydrolysis conditions suggests an N-glucuronide. The partial hydrolysis by arylsulfatase may be due to the unspecificity or impurity of this enzyme or to formation of some N-sulfate. Characteristically DMI and DDMI, but not the tertiary amine IP, are detected as aglycones. The only functional group of DMI or DDMI which can possibly serve as an acceptor for conjugants such as glucuronic acid is the secondary or primary amino group in the aliphatic side chain. N-acetyl-DMI would be extracted with the unconjugated metabolites and can therefore be ruled out as conjugate. The new conjugate of DMI (and DDMI) has not been observed to be formed in humans, rats or guinea-pigs but only in dogs. Similar species differences with respect to formation of N-glucuronides have recently been reported[7]. Information of N-glucuronides is scarce, and knowledge of their properties incomplete. The well-documented cases of N-glucuronide formation have involved aromatic amines, carbamates, and sulfonamides. The only mention of aliphatic N-glucuronides we are aware of is that relating to dogs[8] and humans[9, 10] with mono- and di-demethylated chlorpromazine, i.e., aglycones with structures very similar to DMI and DDMI. However, no details of their identification and properties have been given by these authors.

Table II. Thin layer chromotography of desmethylimipramine and metabolites

Solvent Nr.	Reference	DMI		DDMI		2-OH-DMI	
		Rf	color	Rf	color	Rf	color
I	1	0.43	blue	0.56	blue	0.23	red
II	3	0.53	blue	0.60	red	0.38	red
III	5	0.97	blue	0.30	blue	0.11	red
IV	6	0.69	green	0.82	green	0.12	red
V	5	0.54	blue	0.73	blue	0.55	red

I, chloroform/n-propanol/conc. ammonia 50:50:1; II, benzene/ethyl acetate/ethanol/conc. ammonia 20:20:10:1; III, acetone/ammonia 1*N* 50:50; IV, toluene/chloroform/diethylamine 18:33:9; V, n-butanol/acetic acid/water 60:15:25.

Table III. Percent aglycones formed by hydrolysis under various conditions of bile from dog B

Enzyme	pH	°C	h	2-OH-DMI	DMI
–	2.0	37	1	2	1.5
–	2.0	60	1	2	10
–	2.0	100	1	4	100
–	5.4	37	24	trace	trace
Arylsulfatase	5.4	37	24	trace	25
β-glucuronidase	5.4	37	24	100	100

Zusammenfassung. Nach Verabreichung von Imipramin oder Desmethylimipramin bilden Hunde neben den bekannten Metaboliten Konjugate von Desmethylimipramin (und in kleineren Mengen von Desdimethylimipramin). Die Befunde sprechen für aliphatische N-Glukuronide und damit für einen bisher kaum beschriebenen Typus von Arzneimittelmetaboliten.

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