activation to result in centrilobular lesion while those detoxified directly produce periportal damage. Those toxins which result in detoxification through intermediates which are more toxic than the parent compound and its ultimate metabolites, produce midzonal necrosis[16].

There is now considerable evidence to show that carbon tetrachloride needs to be activated by microsomal enzyme system in the liver before becoming toxic[17,18]. Administration of glucocorticoids increases toxicity of carbon tetrachloride[19] indicating an effective stimulation of drug metabolism during cortisone therapy. Such a treatment in acute aflatoxicosis, on the other hand, results in protection against liver damage[20].

Pregnancy depresses metabolism of various foreign compounds[21] and increases susceptibility to acute aflatoxin damage[22]. Similarly, vitamin A deficiency has been shown to depress the metabolism of a variety of drugs[23] and has been demonstrated to increase the susceptibility to acute aflatoxin damage[24]. A more direct and clinching evidence for the role of metabolism of aflatoxin as a factor determining its toxicity, is provided by the studies on hypophysectomized rats. Hypophysectomy reduced the metabolism of aminopyrin and of aflatoxin[25]. There was a decreased formation of the metabolic products of aflatoxin produced by the microsomal enzymes[25]. However, the acute susceptibility to aflatoxin was greatly increased by hypophysectomy[26].

These data therefore strongly suggest that an effective drug metabolizing system protects the animal from the acute effects of aflatoxin. On the other hand, this effective drug metabolizing system would appear to have exactly the opposite effect on the carcinogenic potential of aflatoxin. Protein deficiency and vitamin A deficiency increase the acute susceptibility, but reduce the incidence of hepatomas[27,28]. Again, hypophysectomy markedly reduces the LD_{50}[26], but is not compatible with the production of liver tumours[29]. These results indicate that during the metabolism of aflatoxin in the body, compounds which are less toxic with respect to acute lesions but more effective in hepatocarcinogenesis are formed. This possibility is supported by the observation that administration of a single large dose of 500 µg of the toxin results in the development of hepatomas in 50% of rats[30], while a smaller dose of less than 100 µg when administered in multiple divided doses over a long period results in liver cancer in 100% of animals[31].

The observation that simultaneous administration of phenobarbitone and aflatoxin reduces the incidence of aflatoxin-induced hepatic tumours, however, suggests that aflatoxin is detoxified with respect to its carcinogenic potential also[32]. This could have resulted from the competitive inhibition of the metabolism of the carcinogen administered while the drug-metabolizing system is handling the phenobartitone.

It appears, therefore, that the microsomal drug metabolizing enzymes are rate limiting in the degradation of aflatoxin in the rat liver. It is likely that these 'detoxified' products are more carcinogenic to the liver. Further studies are necessary to substantiate this hypothesis.

Summary. The evidences to incriminate the possible role of microsomal drug metabolizing system in aflatoxin toxicity in rat have been reviewed. It is suggested that an effective drug metabolism results in decreased acute toxicity and the products of metabolism are more carcinogenic than the native toxin to the rat liver.

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Benzodiazepines: a Comparison of their Effects in Mice on the Magnitude of the Palmar Skin Conductivity Response and on Pentylenetetrazole-Induced Seizures

We have previously demonstrated[1] that a wide variety of CNS depressants inhibit the palmar skin conductivity response in mice (PSCR-test). This test might thus be used to study non-specific sedative activity. On the other hand, the blocking of pentylenetetrazole-induced seizures (anti PIS = APIS-test) at very low doses is characteristic of benzodiazepines and might be related to the anti-epileptic and anxiolytic actions of these drugs. Consequently, a systematic comparison between the two methods deserves investigation.

Methods. Swiss Or1 male mice (body weight 18 to 25 g), randomized into batches of 10, were used for both methods. PSCR- and APIS-tests were carried out following[1] and[2] respectively. The PSCR-test is based on the increase of the palmar skin conductivity level in response to a photostimulus; 13 benzodiazepines and 2 other drugs (cf. Table) were injected i.p. 20 min before the i.p. administration of 125 mg/kg of pentylenetetrazole in the APIS-test or before the 2nd photostimulus in the PSCR-test. Each batch of mice was dosed in such a way as to achieve a logarithmic increase of dosage over the whole experiment. For each drug tested the following values were calculated: the ED 50 (following[3]) preventing tonic convulsions and

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Comparison of the activities in the palmar skin conductivity (PSCR) and antipentylenetetrazole (APIS) tests

Drugs	Activities		APIS A
	APIS A	PSCR A	PSCR A
Clonazepam (cmc)	2695 (2558–2855)	351 (325–375)	7.7 (6.8–8.8)
Flunitrazepam (cmc)	3588 (3187–4158)	514 (513–517)	7.0 (6.5–8.1)
Bromazepam (cmc)	671 [a]	316 (281–348)	2.1
Lorazepam (cmc)	1070 (1048–1101)	664 (623–738)	1.6 (1.4–1.8)
Flurazepam (cmc)	518 [a]	325 (299–351)	1.6
Demoxepam (cmc)	451 (441–463)	286 (265–306)	1.6 (1.4–1.7)
Chlorazepate (cmc)	432 (428–437)	287 (256–318)	1.5 (1.3–1.7)
Tetrazepam (cmc)	388 (374–410)	261 (224–298)	1.5 (1.3–1.8)
Medazepam (sal)	399 (391–409)	290 (263–317)	1.4 (1.2–1.5)
Nitrazepam (cmc)	813 (775–863)	598 (580–628)	1.4 (1.2–1.5)
Oxazepam (cmc)	441 (419–477)	371 (348–392)	1.2 (1.1–1.4)
Diazepam (cmc)	620 (569–724)	542 (528–562)	1.1 (1.0–1.4)
Chlordiazepoxide (cmc)	373 (357–398)	387 (367–405)	1.0 (0.9–1.1)
Phenobarbital Na (sal)	325 (322–329)	343 (324–361)	0.9 (0.89–1.01)
Meprobamate (sal)	283 (277–290)	300 (273–326)	0.9 (0.8–1.1)

Figures in brackets = confidence limits p 0.05.
sal, in Na Cl solution; cmc, in aqueous solution of 0.5% carboxymethylcellulose. [a] Approximate value.

death in 50% of the mice (APIS PD 50 mg/kg), the dose producing a 50% inhibition of PSCR (PSCR ID 50 mg/kg), the corresponding activities:

$$\frac{10^3}{\log (\text{APIS PD } 50 \times 100)} (= \text{APIS A}) \text{ and } \frac{10^3}{\log (\text{PSCR ID } 50 \times 100)}$$

(= PSCR A) and the ratio $\frac{\text{APIS A}}{\text{PSCR A}}$.

The results are given in the Table.

Discussion. Since the various compounds showed greatly differing potencies in the two tests, APIS and PSCR-tests are likely to indicate two different pharmacological actions. The mechanism of the activity of benzodiazepines in APIS-test probably involves an antagonism of the pentylenetetrazole-induced decrease of the presynaptic inhibition in the reticular formation[4]. In the PSCR-test, the effect of benzodiazepines is likely to be the consequence of activity on the ascending reticular formation[5], on autonomic centres and pathways and also on visual pathways[6] as PSCR is elicited by a photostimulus. It is therefore no surprise to find that drugs exhibit different activities in these two tests. So specifically 'anticonvulsant' benzodiazepines (flunitrazepam and clonazepam) reveal an activity ratio APIS A/PSCR A of 7. However, 'sedative' benzodiazepines present an activity ratio which is either around 1, like meprobamate and phenobarbital, or close to 1.5.

Résumé. La comparaison des activités de 13 benzodiazépines sur les tests de réponse de conductivité cutanée palmaire et antipentétrazole permet de différencier par leurs rapports d'activité les benzodiazépines «sédatives» (1,0 à 1,6) et «antiépileptiques» (7).

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Cardiotonic Activities of 3,5-Seco-4-Nor-Cardenolides in *Rana nigromaculata*

In the course of our studies[1–5] on the structure-activity relationship of the cardenolide, several 3,5-seco-4-nor-cardenolides were prepared from digitoxigenin (I), and their cardiotonic activities were tested by using the isolated frog heart (Straub's preparation). In this preliminary report, we describe the cardiotonic activities of the following 4 compounds[6] in comparison with that of digitoxigenin: 14-hydroxy-3,5-seco-4-nor-5-oxo-14β-card-20(22)-enolid-3-oic acid (IIa), its methyl ester (IIb), 5β,14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (III), 5α,14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (IV).

The method of assay is the same as described in the previous papers[1–5]. Frogs, *Rana nigromaculata*, were used. The Straub's cannula contained 2 ml of Ringer's solution, the composition of which was: NaCl, 111 mM; KCl, 2.7 mM; $CaCl_2$, 1.8 mM; $NaHCO_3$, 15 mM, and glucose, 2.7 mM. It was aerated with 95% O_2 + 5% CO_2. The contraction of the heart was recorded with isotonic lever on smoked drums. The heart was first made hypodynamic by reducing the concentration of calcium to 0.6 mM, $^1/_3$ of the normal, and then the effect of one of the compounds was tested in the following way.

Stock solutions of the 5 compounds were prepared with 95% ethanol in concentration of 1 mg/ml. Before experiment, these stock solutions were diluted with 0.6% saline to the desired concentrations. Starting from a subthreshold dose, a small amount (20-140 µl) of a diluted solution was added to the cannula every 15–25 min, so that a stepwise increase in the cumulative concentration of the test

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