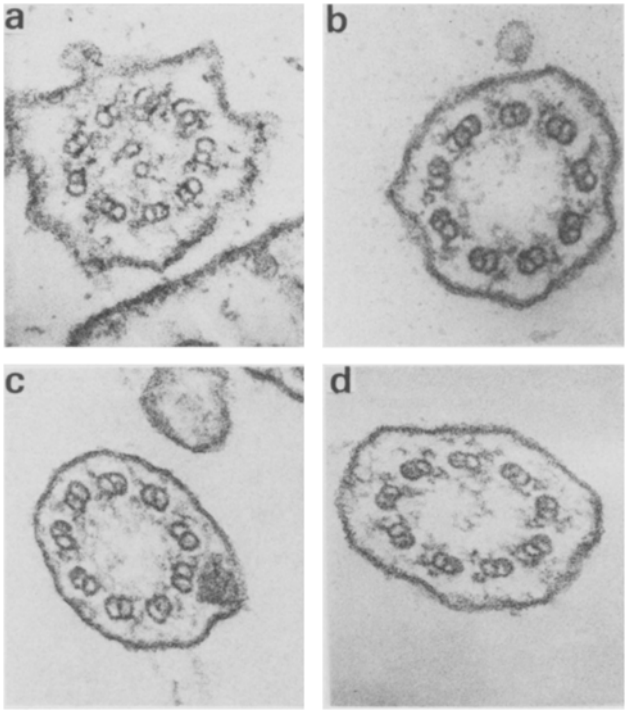
Japan, and Southeast Asian horseshoe crabs, *Tachypleus gigas* and *Carcinoscorpius rotundicauda,* were kindly provided by Dr Smarn Srithunya (Zoological Museum, Srinakharinwirot University, Thailand). American horseshoe crabs, *L. polyphemus,* which were reexamined, were supplied from the Department of Marine Resources, Marine Biological Laboratory, Woods Hole, Mass., USA. The spermiducts of the 4 species were fixed in 2% glutaraldehyde, buffered with 0.2 M sodium cacodylate (pH 7.4) and containing 7% sucrose, for 1 h, washed with the buffer for 3 h and postfixed in 2% osmic acid in the same buffer for 1 h. Then the specimens were dehydrated in an ethanol series and embedded in Spurr Resin. Ultrathin sections were cut with glass knives, stained with uranyl acetate and lead nitrate, and observed in a JEOL's JEM 100-C electron microscope. In contrast with the axoneme of *L. polyphemus* (fig., a), which has the 9+2 pattern, the 3 Asian species had a 9+0 pattern without central tubules (fig., b, c, d). These spermatozoa were motile. Costello suggested that 9+2 spermatozoa have a planar motion, while 9+1 or 9+0 spermatozoa have a helical one[9,10]. We are studying the details of the motion of the spermatozoa, the ultrastructure of their other organellae and the species specific interaction of spermatozoa with eggs. Baccetti reported that among arthropods only *Limulus* has the basic aquatic sperm whose flagella have the usual 9+2 pattern, without other accessory fibrils, and he proposed a monophyletic origin of arthropods, starting from a *Limulus*-like aquatic ancestor[3]. On the basis of comparative studies on the sperm, he proposed various common evolutionary pathways in Arthropoda with similar steps, whose first step is the acquisition of unusual axoneme patterns[3]. According to his hypothesis and our observations, Asian horseshoe crabs appear to have evolved further than *L. polyphemus.* This idea seems to be consistent with data on fossils suggesting that Limulinae and Tachypleinae branched off in the Jurassic period[11], and also consistent with the results of hybridization experiments[12], the constitution of hemocyanin monomers[13] and phylogenetic analyses based on amino acid sequences of coagulogens[14].



Axoneme patterns of horseshoe crabs. *a Limulus polyphemus* (9+2); *b Carcinoscorpius rotundicauda* (9+0); *c Tachypleus tridentatus* (9+0); *d Tachypleus gigas* (9+0). × 112,000.

1 Contribution No. 40 from the Shimoda Marine Research Center. Supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.
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Diphasic action of 2,2-diphenylpropionic acid N,N-diethylaminoethyl ester hydrochloride on hepatic drug metabolism in the mouse

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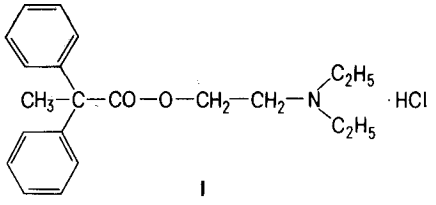
Summary. 2,2-Diphenylpropionic acid N,N-diethylaminoethyl ester hydrochloride, 30 min after a single i.p. dose, inhibits the microsomal drug-metabolizing enzymes of the mouse liver and prolongs the hexobarbital sleeping time; 24 h after the administration, it induces hepatic microsomal drug-metabolizing enzymes and shortens hexobarbital sleeping time.

Some organic compounds such as piperonyl butoxide[1], malonic acid derivatives[2], 2-hydroxy-2-ethylbutyryl N,N-diethylamide[3], hexobarbital[4], and diazepam[5], act both as inhibitors and inducers of hepatic drug metabolism. The induction of drug metabolism which occurs after initial drug biotransformation inhibition, is an adaptive process of the liver in order to overcome enzyme inhibition by these substances[6,7]. This paper is concerned with the same diphasic action on drug metabolism exerted by the basic ester, 2,2-diphenylpropionic acid N,N-diethylaminoethyl

ester hydrochloride ('aprophen', I) which has already been shown to inhibit hepatic microsomal mixed-function oxidases in vitro[8]. This compound is a lower homologue to the compound SKF 525-A (2,2-diphenylvalerianic acid N,N-diethylaminoethyl ester hydrochloride) which is known to inhibit hepatic drug-metabolizing mixed-function oxidases both in vivo[9] and in vitro[10,11].



I

Materials. Inorganic salts, buffer compounds, reagents, and solvents were purchased from Merck AG (Darmstadt, FRG) and Riedel-de Haën (Seelze, FRG), biochemicals from Boehringer (Mannheim, FRG) and Calbiochem-Behring GmbH (Giessen, FRG), hexobarbital (as the sodium salt, Evipan®-Natrium) from Bayer AG (Leverkusen, FRG), aminopyrine and 4-methoxybiphenyl from Hoechst AG (Frankfurt, FRG), 2,2-diphenylpropionic acid from EGA Chemie (Steinheim/Albuch, FRG) and from Aldrich-Europe (Beerse, Belgium), ketamine (Vetalar®) from Parke, Davis & Co. (München, FRG), 7-methoxy-4-methylcoumarin ('methylayapanine') from Calbiochem-Behring GmbH (Gießen), and N,N-diethylaminoethanol from Merck AG (Darmstadt).

Methods. Aprophen was synthetized from 2,2-diphenylpropionic acid and N,N-diethylaminoethanol by the method of Zaugg and Horrom[12].

The experimental animals, male mice of the NMRI strain (b.wt 20 ± 1.5 g) which had free access to food and drinking water, were allocated, at random, to the 3 groups. The 1st group served as the control group, the animals of the 2nd group received aprophen, in a single i.p. dose of 50 mg/kg (in 1% solution in physiological NaCl), 30 min before the observation time, and those of the 3rd group received the same i.p. dose of aprophen, 24 h before the observation time. At observation time, 5 mice of each of the 3 groups were killed by exsanguination (after a blow on their heads); their livers were removed quickly and chilled immediately by immerging them into liquid nitrogen; they were stored in the frozen state at −20 °C, in the refrigerator until further handling. At the same point of time, the rest of the animals of the 3 groups were injected i.v., 100 mg/kg hexobarbital for the measurement of hexobarbital sleeping time[13].

After thawing, the liver specimens were homogenized in ice-cold isotonic saccharose solution with a homogenizer of the Potter-Elvehjem[14] type, and microsomes were prepared by the $CaCl_2$ precipitation method[15]. In these microsomes, the activity of ketamine N-demethylase was assayed as described below, those of aminopyrine N-demethylase by the method of Leber et al.[16], those of methylayapanine O-demethylase as described elsewhere[17], those of methoxybiphenyl O-demethylase as described before[18], and the activities of microsomal, $NADPH_2$-dependent cytochrome c reductase by the method of Cleveland and Smuckler[19].

The ketamine N-demethylase assay mixture contained, in a total volume of 3.0 ml, 200 µmoles of tris(hydroxymethyl)-aminomethane HCl buffer (pH 8.0, previously saturated with air by bubbling air through the buffer solution), 10 µmoles of $MgSO_4$, 9 µmoles of ketamine, 0.10 ml of a $NADPH_2$-regenerating system[17], and 0.50 ml of the microsomal suspension in isotonic KCl solution corresponding to 100 mg of liver wet weight. The enzyme action was started by the addition of the microsomal suspension, and, after incubation for 30 min at 37 °C in open test tubes, was stopped by the addition of 1.0 ml of 10% trichloroacetic acid solution. In the corresponding reference incubation, the trichloroacetic acid solution was added immediately after the addition of the microsomal suspension. After centrifugation, the formaldehyde formed by the enzymic demethylation was measured by the Nash[20] procedure as described by Cochin and Axelrod[21]. Under these assay conditions, reaction rates are proportional to time and enzyme content, as shown before (unpublished results).

Results and discussion. The results are listed in the table. 30 min after the administration of the drug, hexobarbital sleeping times are increased and the enzyme activities decreased significantly, as calculated with the t-test[22]. 24 h after the application, hexobarbital sleeping times decreased and enzyme activities elevated significantly, with the exception of methylayapanine O-demethylase, which shows no significant change. All these results are comparable with each other because both hexobarbital sleeping time determinations and sacrifice for enzyme activity determination had been done at the same time for all 3 groups of the mice, so that neither diurnal rhythms nor time delays influence the results. There is an inhibition of drug metabolism, 30 min after the administration of aprophen as shown both by the decreased demethylase activities and the decreased rate of hexobarbital metabolization (expressed as sleeping time prolongation[4], and an induction of drug metabolism, 24 h after the administration of 2,2-diphenylpropionic acid N,N-diethylaminoethyl ester hydrochloride as shown by increased enzyme activities and by increased hexobarbital metabolization (visualized by shortened sleep-

Hexobarbital sleeping times and liver enzyme activities in mice treated with 2,2-diphenylpropionic acid N,N-diethylaminoethyl ester hydrochloride

	Control (%)	30 min before	24 h before
Hexobarbital sleeping time	92.2 ± 30.2	393.9 ± 85.7	31.5 ± 10.6
(min)	(100.0 ± 32.8)	(427.1 ± 92.9)	(34.1 ± 11.4)
Ketamine N-demethylase	0.122 ± 0.017	0.095 ± 0.014**	0.176 ± 0.013
(U/g liver)	(100.0 ± 13.9)	(77.9 ± 11.5)	(136.9 ± 10.7)
Aminopyrine N-demethylase	0.231 ± 0.023	0.124 ± 0.015	0.326 ± 0.012
(U/g liver)	(100.0 ± 10.0)	(53.7 ± 6.5)	(141.1 ± 5.2)
Methoxybiphenyl O-demethylase	0.278 ± 0.034	0.172 ± 0.012	0.358 ± 0.019
(U/g liver)	(100.0 ± 12.2)	(61.9 ± 4.3)	(128.8 ± 6.8)
Methylayapanine O-demethylase	0.0531 ± 0.0048	0.0343 ± 0.0019	0.0590 ± 0.0053
(U/g liver)	(100.0 ± 9.0)	(64.6 ± 3.6)	(111.1 ± 10.0)*
Cytochrome c reductase	10.7 ± 0.3	7.9 ± 0.7	12.7 ± 1.0
(U/g liver)	(100.0 ± 3.1)	(73.8 ± 6.5)	(118.7 ± 9.3)

Mean ± SD; N = 5. *Nonsignificant, **$p < 0.05$, all others: $p < 0.005$. Numbers in brackets are percentage of control.

ing times). So far, our results with aprophen are in congruence with those obtained with other compounds[3-5,23], and show that aprophen acts diphasically on hepatic microsomal drug metabolism. But in contrast to those compounds which do not inhibit cytochrome c reductase, the activity of this enzyme is decreased 30 min after aprophen administration. In this respect, 2,2-diphenylpropionic acid N,N-diethylaminoethyl ester hydrochloride behaves like N,N-diethylacetamide in that both demethylases and cytochrome c reductase are decreased shortly after the administration of the compound[24]. In the case of both substances, there is no inhibition of cytochrome c reductase in vitro[8,24]. This means that with aprophen and diethylacetamide, drug metabolism inhibition as found in in vivo experiments is not only confined to an interaction of the drug metabolism inhibitors with cytochrome P-450 as it is observed with 2-hydroxy-2-ethylbutyryl N,N-diethylamide[25] and is discussed for the other drugs mentioned earlier[7], but that there are additional effects of these 2 compounds on microsomal drug-metabolizing enzymes which lead to a decrease of the cytochrome reductase, too, but these additional effects do not occur in vitro. In vitro, aprophen is shown to inhibit mixed-function oxidases but not cytochrome c reductase[8]. This to lesser extent, is the case with the free 2,2-diphenylpropionic acid[26]. N,N-diethylacetamide has no effect at all on microsomal drug metabolism in vitro[24]. The nature and the toxicological consequences of these different effects in vivo and in vitro are being studied.

There is another striking result which only can be pointed to but cannot be discussed at length, namely the lack of inverse proportionality between hexobarbital sleeping time and drug-metabolizing enzyme activity. At first glance, one might expect such an inverse proportionality to occur theoretically. The same lack of inverse proportionality was observed with 2-hydroxy-2-ethylbutyryl N,N-diethylamide[3] and with the action times of a variety of other drugs, too (unpublished results). The proper explanation for these effects have yet to be found although some first approximating steps already exist[27].

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Inhibition of neurotensin (NT)-induced glucagon release by [D-Trp^{11}]-NT[1]

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19 February 1982

Summary. A synthetic analog of neurotensin (NT), [D-Trp^{11}]-NT, antagonized NT-induced hyperglucagonemia and hyperglycemia, and also NT-induced glucagon release from pancreatic islets in rats.

The tridecapeptide neurotensin (NT)[2,3] with the structure of pGlu^1-Leu^2-Tyr^3-Glu^4-Asn^5-Lys^6-Pro^7-Arg^8-Arg^9-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}-OH has been found to be distributed in the brain, gut, pancreas and blood[4-6], and to exhibit a variety of biological effects, including hyperglucagonemia[7-9] and others[10].

Recent structure-activity studies using NT and its analogs have yielded evidence to support the view that the C-terminal part (hexapeptide) of the molecule is essential for the biological activity of NT[11-17] and that the chemical structure of Tyr^{11} may play a critical role in the process of NT receptor activation[13,16,17]. It is of special interest that [D-Trp^{11}]-NT antagonized selectively NT-induced coronary vasoconstriction in rats, but displayed NT-like activity in higher concentrations[17].

The present work was undertaken to investigate the effect