changes were not significant. There were no differences in amylase activity between control and experimental animals.

Secretion of digestive enzymes from pancreas operates via the pathway which is Ca^{2+}-dependent and involves cholinergic and peptide hormone receptors[18,19]. In this study the effect of cycloheximide on the activity of lipase, amylase, trypsin and chymotrypsin present in rat pancreas, as well as circulating in the serum, was measured. To understand the cycloheximide-induced inhibition of exocrine pancreatic enzymes, one has to take into consideration the inhibitory effect of the drug on gastric secretion[13]. The activity of pancreatic enzymes depends on and is modified by end products of digestion appearing in the blood[20–23]. In this way the impaired digestion and the delay of gastric emptying caused by cycloheximide, simulating to some degree starvation of the animals, can indirectly affect the level of exocrine pancreatic enzymes.

Following cycloheximide administration, direct inhibition of enzyme synthesis in acinar cells can also be expected and the interference of the drug with receptors necessary for enzyme secretion cannot be excluded. Decreased activity of lipase and trypsin following cycloheximide administration and significantly decreased pathological changes in histological sections evaluated in experimental pancreatitis following drug treatment (unpublished) present potential opportunities for the use of cycloheximide in gastroenterology.

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[^{3}H]-Norcocaine and [^{3}H]-Pseudococaine: Effect of N-Demethylation and C_2-Epimerization of Cocaine on its Pharmacokinetics in the Rat[1]

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Summary. After i.v. injections of cocaine, norcocaine, pseudococaine to the rat, the $T_{1/2}$ in brain were 0.4, 0.6, 0.2 h respectively and in plasma 0.4, 0.5, 0.2 h respectively. Benzoylnorecgonine and norecgonine were the metabolites of norcocaine in brain. Pseudonorcocaine, pseudobenzoylnorecgonine, pseudobenzoylecgonine and pseudoecgonine were the metabolites of pseudococaine in rat brain. Benzoylnorecgonine and pseudobenzoylecgonine had potent stimulant activity intracisternally in the rats.

Norcocaine (Figure 1) has been reported to be an important metabolite of cocaine in the rat[2,3] dog[4] and monkey[5]. Like cocaine, it is a powerful central nervous system stimulant[6,7] of short duration and it contributes possibly in part to the pharmacological effects of cocaine. Pseudococaine (dextro isomer and C_2-epimer of cocaine) has the C_2-methyl ester group in equatorial configuration and *trans* to the nitrogen and C_3-benzoyloxy side chain[8] (Figure 1). Its toxicity and local anesthetic activity is several-fold higher than cocaine[9–11]. Both isomers have convulsant and paralysant properties[10–14], but pseudococaine is several times less potent than cocaine as an inhibitor of norepinephrine uptake in ventricles and vas deferens slices[13,15]. It has been reported to be more extensively degraded than cocaine[16,17] in cats. No information exists on the dispositional and metabolic profile of norcocaine and pseudococaine in the central nervous system. This study was undertaken to obtain information on these parameters.

Materials and methods. Norcocaine was prepared by a procedure previously described[18]. Pseudococaine was prepared by conversion of cocaine to pseudo ecgonine methyl ester[8] and subsequent benzoylation with benzoylchloride in benzene using standard procedures. Samples (50 mg) of these non-labelled compounds were tritiated (New England Nuclear, Boston, Mass.) by acid-catalyzed exchange tritium labelling using 0.3 ml acetic acid, 25 mg platinum catalyst and 10 curies of tritiated water. The crude products were exhaustively purified in our laboratory by repeated solvent extractions and silica gel column chromatography (eluant, benzene-methanol

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mixtures of increasing polarity). The specific activities and radiochemical purities of [^{3}H]-norcocaine and [^{3}H]-pseudococaine were 2.56 mCi/mg, 96% and 1.97 mCi/mg, 98% respectively. The hydrochlorides of these compounds were suitably diluted with nonradioactive compounds in 0.9% saline to specific activities of 64 and 40 μCi/mg respectively for the preparation of injection solutions.

Male Wistar rats (120–150 g) were injected i.v. with 2 mg kg^{-1} (free base) dose of [^{3}H]-cocaine, [^{3}H]-norcocaine or [^{3}H]-pseudococaine. The brain and plasma at different times were obtained as previously described [19,20]. Free [^{3}H]-norcocaine and [^{3}H]-pseudococaine were determined in brain and plasma by the procedure used for cocaine [2,3]. In vitro recoveries of [^{3}H]-norcocaine and [^{3}H]-pseudococaine were in the range described for cocaine [2,3]. Other details on the specificity of extraction procedure, counting technique, etc., have been previously described [3].

The qualitative identification of metabolites of norcocaine and pseudococaine was done in groups of 5 brains of rats injected with 2 mg kg^{-1} i.v. dose of these compounds. The brains were homogenized in 0.5 *M* HCl (20% homogenate) centrifuged, supernatant removed and its pH adjusted to 7.5 with dilute ammonia. The resultant solution was chromatographed on Amberlite XAD-2 (2 × 15 cm) and the adsorbed compounds and their metabolites eluted with methanol. The residue from this methanol eluate was chromatographed on Gelman instant thin layer chromatography media silica gel (ITLC) with different solvent systems [21] for characterization of metabolites. The solution containing unadsorbed radioactivity from the Amberlite XAD-2 column was saturated with K_2CO_3 and extracted repeatedly with chloroform-isopropanol (2:1, v/v), the organic phase evaporated to dryness in vacuo and the residue therefrom submitted to ITLC for characterization of the metabolites.

Results and discussion. Comparative distribution of [^{3}H]-cocaine, [^{3}H]-norcocaine and [^{3}H]-pseudococaine in rat brain and plasma after a 2 mg kg^{-1} i.v. injection is shown in Figure. 2. The peak concentrations of norcocaine in brain and plasma were approximately 2.3

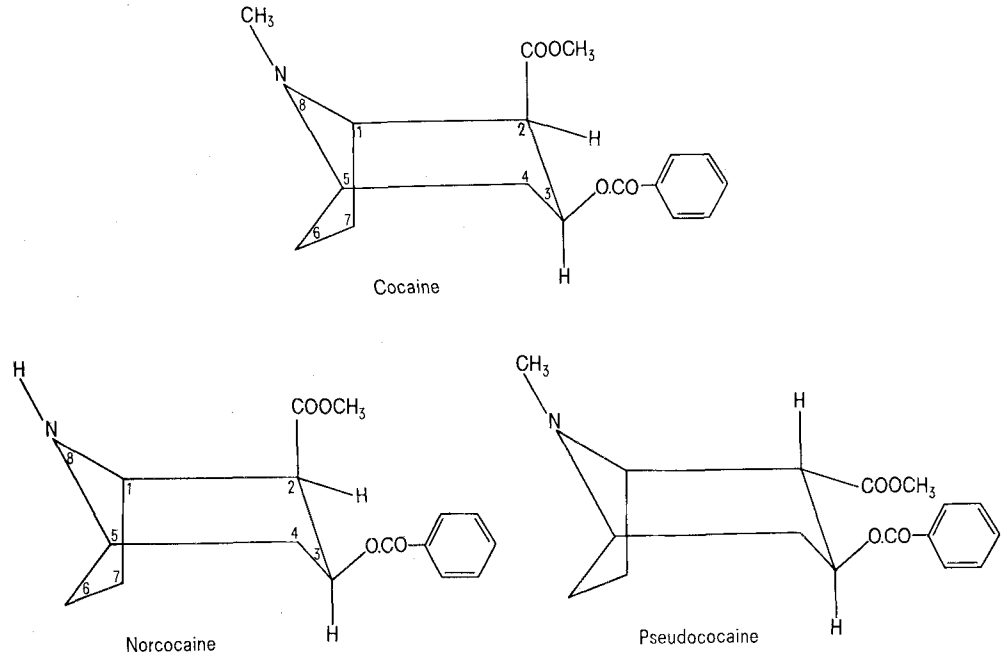

Fig. 1. Structures of cocaine, norcocaine and pseudococaine.

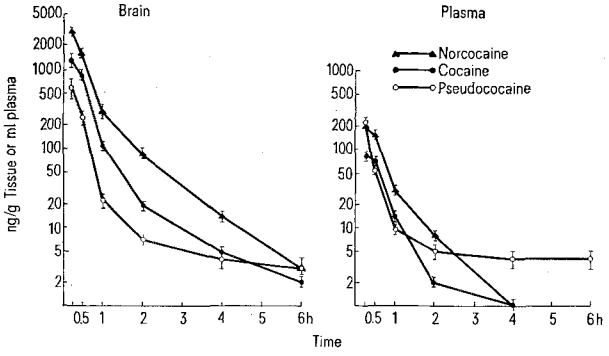

Fig. 2. Comparative distribution of [^{3}H]-cocaine, [^{3}H]-norcocaine and [^{3}H]-pseudococaine in brain and plasma of male Wistar rats after a single 2 mg kg^{-1} (free base) dose by i.v. injection. Data represent mean value ± SEM (ng per g wet tissue weight or ml fluid) of 6 determinations from 3 animals at each time.

times those of cocaine. The apparent partition coefficient of [^{3}H]-norcocaine in 1-octanol-M/15 phosphate buffer, pH 7.4 in the concentration range 1–10 μg/ml at 25 °C was 18.5 ± 0.2 (SEM). Inspite of the high lipid solubility of pseudococaine (apparent partition coefficient in 1-octanol-M/15 phosphate buffer, pH 7.4, 77.2 ± 2.2 (SEM), its peak concentrations in brain were approximately half those of cocaine, while the plasma levels were approximately 2.5 times those of cocaine. Rapid metabolism of pseudococaine led to comparatively lower brain levels. Like cocaine, norcocaine and pseudococaine did not persist in the CNS and the levels in brain and plasma were barely detectable 6 h post-injection. The half-lives of

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cocaine, norcocaine and pseudococaine in rat brain were 0.4, 0.6 and 0.2 h respectively; that in plasma 0.4, 0.5 and 0.2 h respectively. The brain to plasma ratios 0.25, 0.5, 1, 2, 4 h post-injection were as follows: cocaine 15.9, 11.5, 7.9, 9.5, 5; norcocaine 15.7, 10.6, 9.7, 10.9, 14; pseudococaine 2.8, 4.7, 2.4, 1.4, 1.0 respectively.

Thin layer chromatographic experiments on brain extracts (30 min) of rats injected i.v. with norcocaine provided evidence for the presence of major amounts of norcocaine (72%), benzolnorecgonine (24%) and minor amounts of norecgonine (4%). Benzoylnorecgonine has earlier been shown[6] to possess potent stimulant activity intracisternally (i.c.) in the rat and formed molecular complex[22] with Ca^{2+}. Similar experiments with pseudococaine provided evidence for the presence in brain of pseudonorcocaine (12%), pseudobenzoylnorecgonine (12%), pseudobenzoylecgonine (8%) and pseudoecgonine (minor amounts) as metabolites of pseudococaine (66%).

Our preliminary studies on the pharmacological activity of these metabolites showed that pseudoecgonine and pseudoecgonine methyl ester (1 mg kg^{-1} i.c.) showed no activity in the rat. Pseudobenzoylecgonine at this dose produced intermittent convulsions lasting approximately 15 sec and death within 10 min; lower doses 0.5 mg kg^{-1} (i.c.) produced rapid heart beat, laboured breathing, running activity, shivering, disorientation, jerking and convulsions 4 min post-injection lasting until death 30 min later. A 0.25 mg kg^{-1} dose (i.c.) produced similar effects without mortality. Pseudococaine (0.5–1 mg kg^{-1} i.c. rats) produced rapid heart beat, laboured breathing, gasping and death within 1 min and lower doses produced similar effects without mortality. Norcocaine (i.v.) was approximately 2 to 3 times more potent a stimulant as compared to cocaine.

This study demonstrates that N-demethylation and C_2-epimerization of cocaine lead to compounds which have significantly different dispositional profile in the CNS, and have more potent stimulant activity as compared to cocaine. In addition, some of the polar metabolites of norcocaine and pseudococaine, e.g. benzoylnorecgonine and pseudobenzoylecgonine, possessed potent stimulant activity intracisternally in the rat.

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On the Mechanism of Pyrogenic Action of Ricin

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Summary. The incubation of rabbit white blood cells with ricin, the toxic protein of castor oil seeds, leads to the production of endogenous pyrogens. This induction can be inhibited by the antibiotics actinomycin D or cycloheximide. The results are discussed in terms of disturbed corticosteroid- and Mg^{2+}-levels.

The authors completely agree with the suggestion that all of the known pyrogenic substances cause fever on a common basis, i.e., by the induction of the so-called 'endogenous or leukocytic pyrogen'[3–5].

According to this experimentally supported hypothesis, the pyrogens, during a relatively short period of a few hours, activate the different cells of the organism. These cells, mainly the leukocytes[6, 7], the monocytes[8], and the Kupffer's cells of the liver[5], also release endogenous pyrogens in the so-called 'early' and 'late' phases into the blood stream. During this process, the cells show different metabolic changes, e.g., increased oxygen consumption, carbon dioxyde production and increased glycolysis. This endogenously released pyrogen induces fever by acting on the central nervous system[5].

It has been experimentally demonstrated and accepted that, during the so-called 'early' phase, synthetic processes, mainly protein and RNA synthesis, take place[9]. In vitro these early processes can be inhibited by substances having an inhibitory effect on protein synthesis, for example, actinomycin-D, cycloheximide and puromycine[5, 10], while the processes of the 'late' phase are resistant to these drugs.

In recent years it has been convincingly shown that ricin has a powerful pyrogenic effect on different animals[11]. The fever caused by ricin is very high and of long duration in all species, which is similar to the fever caused by the true bacterial pyrogens[3]. Therefore it is likely that ricin also causes fever by the release of endogenous pyrogen substances. If the 'early' phase of pyrogen release of the leukocytes activated by ricin is inhibited by actinomycin-D or another drug with a similar effect, this will lend considerable support to this possibility. To investigate this, the following experiments were done.

Materials and methods. All needles, syringes and solutions were sterile and pyrogen-free. Living white blood cells were obtained under sterile conditions from the blood of healthy rabbits by heart puncture. At the time of collection, 10 IU/ml heparin was added to the blood to inhibit clotting. This quantity of heparin has no effect on the endogenous pyrogen production[12]. After 25 min of centrifugation (3000 rpm, on 4 °C), the white blood cells were aspirated away carefully with a pipette and suspended

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