Another aim of our study was to see whether agents capable of curing α-amanitin poisoning[4,14,15] are also effective against phalloidin. Out of this category neither penicillin nor cytochrome C protected against phalloidin. So antagonism to amatoxins does not extend to phallotoxins. This is in line with the contention that the two classes of poisonous principles in *Amanita phalloides* act by different mechanisms. On the other hand, some agents protecting against phalloidin such as carbon tetrachloride are not completely devoid of activity against α-amanitin[3]. No effect was seen with activated charcoal, tested in the light of the report indicating a hepatoenteric circulation of *Amanita* poisons[16].

A substantial protection against phalloidin was provided by cysteamine (β-mercaptoethylamine). This agent was effective in the treatment of experimental[17,18] and clinical[19] paracetamol poisoning, perhaps by scavenging the active toxic paracetamol metabolite thought to combine with vital liver-cell macromolecules and held responsible for the ensuing liver injury. Although cysteamine might inhibit the formation of a toxic phalloidin metabolite, it is known to prevent the interaction of drug metabolites with liver proteins. This opens a new vista on the mechanisms by which phalloidin toxicity may be antagonized. In current studies, agents antagonizing the single toxins phalloidin and α-amanitin are tested against total extracts of *Amanita phalloides*.

Zusammenfassung. Der Schutzeffekt von Rifampicin gegen eine Letaldosis des Pilzgiftes Phalloidin wurde bestätigt und weiter charakterisiert. Auch Cysteamin erwies sich als wirksam. Keinen Schutz gegen Phalloidin boten Stoffe, welche das Pilzgift α-Amanitin antagonisieren. Die hepatotoxische Wirkungskomponente der meisten Phalloidin-Antagonisten wird erörtert.

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Identification of Norcocaine as a Metabolite of [^{3}H]-Cocaine in Rat Brain

Cocaine is a powerful central nervous system stimulant of relatively short duration, low margin of safety, high systemic toxicity (LD_{50} i.v. in rats 17.5 mg/kg) and little recognized medical use. Its intense euphoriant action leads to a very high degree of psychic dependence[1,2] and a profound and dangerous type of drug abuse. Physical dependence and tolerance to its euphoria and toxic effects has, however, not been reported to develop. In spite of much work[3–14], the dispositional and metabolic profile of cocaine in the central nervous system remains unknown. This study deals with these parameters and demonstrates that: a) cocaine disappeared fairly rapidly from the rat brain after s.c. and i.v. injections; b) in addition to the formation of benzoylecgonine, benzoylnorecgonine and ecgonine as metabolites, N-demethylation occurs rapidly in the brain to form norcocaine, a lipophilic compound with convulsant properties similar to those of cocaine.

Materials and methods. Male Wistar rats (120–150 g) were injected s.c. with 20 mg kg^{-1} (free base) dose or i.v. with 8 mg kg^{-1} (free base) dose of [^{3}H] cocaine prepared as described previously[15] (radiochemical purity 98%,

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Uptake of [^{3}H] cocaine in brain and plasma of male Wistar rats after a single 8 mg kg^{-1} (free base) i.v. or 20 mg kg^{-1} (free base) s.c. injection

	0.25 h	0.5 h	1 h	2 h	4 h	6 h	12 h	24 h	Half-life (h)
Brain (i.v.)	7269 ± 177	2738 ± 634	924 ± 131	212 ± 48	8 ± 3	0	—	—	0.4
Plasma (i.v.)	612 ± 62	296 ± 90	111 ± 14	26 ± 8	0	—	—	—	0.3
B/P[a] (i.v.)	11.9	9.3	8.3	8.1	—	—	—	—	
Brain (s.c.)	—	2237 ± 282	2320 ± 205	2963 ± 195	3439 ± 362	485 ± 194	4 ± 1	0	4.8
Plasma (s.c.)	—	249 ± 51	378 ± 31	448 ± 74	494 ± 59	78 ± 35	2 ± 1	0	5.0
B/P[a] (s.c.)	—	9.0	6.1	6.6	6.9	6.2	2	—	

Data represent the mean value ± S.E.M. (ng/g of tissue or ml of fluid) of 3 animals for i.v. and 5 animals for s.c. group at each time period.
[a] B/P represents the ratio of mean brain to plasma concentrations.

specific activity 12 μCi/mg). The brains and plasma at different periods of time were obtained as previously described[16–19]. Free [^{3}H] cocaine was determined by homogenization of brain in 0.5 *M* HCl (10% homogenate), adjusting the pH of a 2 ml aliquot of homogenate or diluted plasma (1:5) containing 1 ml nonradioactive cocaine hydrochloride carrier (500 μg/ml) with dilute ammonia to pH 8–9; this was buffered with 1 ml of 20% K_2HPO_4 solution and extracted with 15 ml cyclohexane by shaking for 15 min and the organic phase was centrifuged for 10 min, washed with 4 ml 0.1 *M* NaOH solution. The residue obtained by evaporating 10 ml aliquots of organic phase in counting vials was dissolved in 0.5 ml isopropanol and radioactivity counted with 10 ml toluene-phosphor solution. In vitro recoveries of [^{3}H] cocaine from tissue homogenates and fluids by this method were quantitative in the concentration range 1–1000 ng and the minimal amount of cocaine detectable was 1 ng. The method did not extract benzoylecgonine, benzoylnorecgonine and ecgonine, but norcocaine, a minor metabolite was extracted. A rough estimate of the amount of norcocaine present in rat brain 3.5 h following a 20 mg kg^{-1} s.c. dose was 10% of the extracted value.

The qualitative identification of [^{3}H] cocaine and its metabolites was done on groups of 5 brains removed 3.5 h after a single s.c. injection of 20 mg kg^{-1} dose. The brains were homogenized in 0.5 *M* HCl (20% homogenate), centrifuged to remove debris, the pH of the supernatant adjusted to 7.5 and chromatographed on Amberlite XAD-2 The adsorbed cocaine and its metabolites were eluted with methanol and the residue from the eluate was chromatographed[20] on ITLC (silica gel) with 4 solvent systems and chromatograms radioscanned. The brain homogenate supernatant containing unadsorbed radioactivity from Amberlite XAD-2 column was adjusted to pH 10–12 with dilute NaOH and extracted repeatedly with 12 times its volume of chloroform-methanol (68:32, v/v). The filtered organic extract was evaporated to dryness in vacuo and the residue was chromatographed on ITLC with solvent systems: S_1 (Figure g), S_2 and S_4, respectively and paper chromatographed with *n*-butanol-acetic acid-water (4:1:5, v/v).

Results and discussion. The mean values on the uptake of [^{3}H] cocaine by rat brain and plasma at different time intervals after a single s.c. injection of 20 mg kg^{-1} dose or

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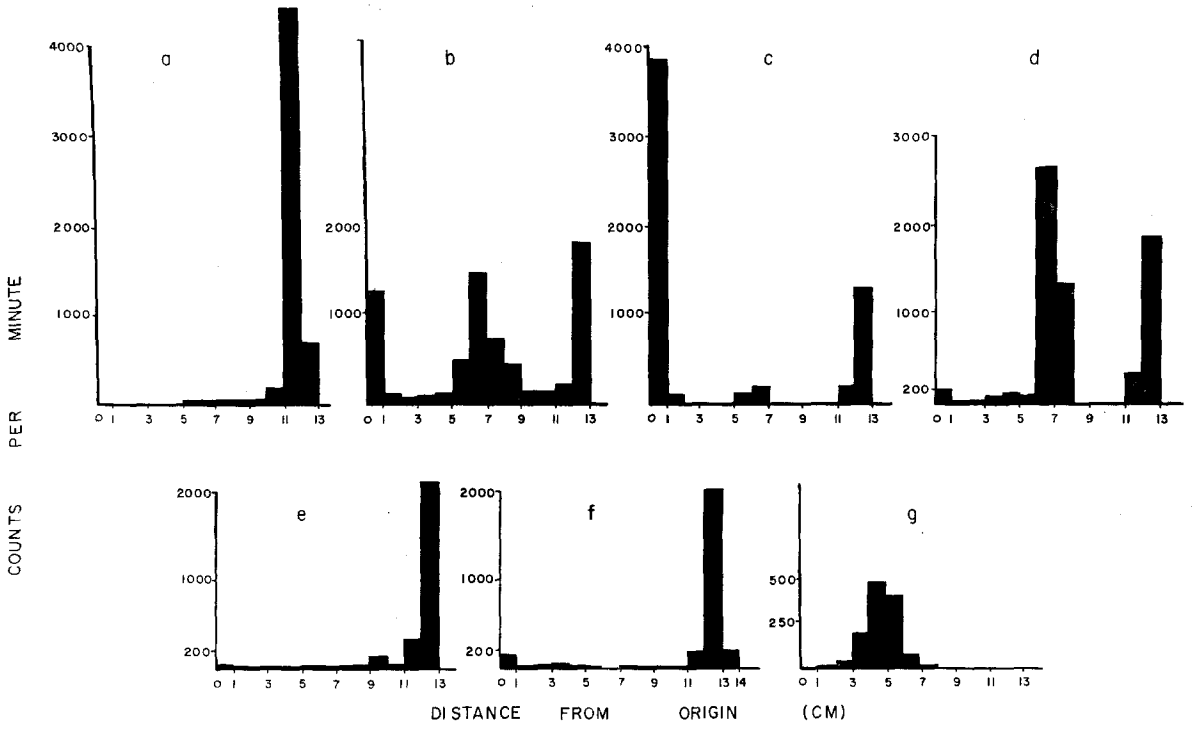

Radioscans of thin-layer chromatograms (silica gel) of brains of rats injected s.c. with 20 mg kg^{-1} dose of [^{3}H] cocaine, removed 3.5 h after injection, processed on Amberlite XAD-2 resin, methanol-soluble residue from Amberlite XAD-2 resin in a) solvent system S_1: *n*-butanol-acetic acid-water (35:3:10, v/v) a single peak of radioactivity Rf 0.88; b) in solvent system S_2: chloroform-acetone-diethylamine (5:4:1, v/v), 3 peaks of radioactivity Rf 0.05 (benzoylnorecgonine), 0.5 (benzoylecgonine), 0.96 (cocaine and norcocaine) with distribution of radioactivity as 20, 49, 31%, respectively; c) in solvent system S_3: *n*-hexane-ethyl acetate-concentrated ammonia (60:40:0.1, v/v), 3 peaks of radioactivity Rf 0.05 (benzoylnorecgonine, benzoylecgonine), 0.55 (norcocaine), 0.96 (cocaine) with distribution of radioactivity as 66, 8, 26%, respectively; d) in solvent system S_4: ethyl acetate-methanol-concentrated ammonia (17:2:1, v/v) 4 peaks of radioactivity Rf 0.05 (unknown), 0.35 (benzoylnorecgonine), 0.55 (some benzoylnorecgonine and benzoylecgonine), 0.96 (cocaine and norcocaine) with distribution of radioactivity as 3, 4, 61 and 32%, respectively; e) separation of norcocaine and cocaine on ITLC (silica gel), solvent system S_3, Rf norcocaine and cocaine 0.72, 0.96, respectively; f) ITLC (alumina) in solvent system benzene-ether (8:2, v/v), Rf norcocaine, cocaine 0.05–0.3 and 0.9 respectively; g) ITLC of the organic solvent extract of brain homogenate from Amberlite XAD-2 column containing unadsorbed radioactivity, in solvent system S_1, a single peak of radioactivity due to ecgonine Rf 0.43.

8 mg kg^{-1} i.v. injection dose are given in the Table. The radioscans (details given in Figure a–d) provided evidence for 4 metabolites which were separately eluted by previous techniques[16–19] and characterized by co-chromatography with standard samples[21], as benzoylnorecgonine, benzoylecgonine, norcocaine and an unidentified metabolite. Details of separation of norcocaine from cocaine on ITLC (silica gel) and ITLC (alumina) are shown in Figure e–f. Norcocaine was also detected as a metabolite of cocaine in rat brains within 1 min of onset of convulsions following a 10 mg kg^{-1} i.v. dose of [^{3}H] cocaine. A single peak of radioactivity in solvent systems S_1 (Figure g), S_2, S_4 and paper chromatogram and co-chromatography with standard ecgonine confirmed the identify of Amberlite XAD-2-unadsorbed metabolite as ecgonine. The brain debris remaining after the extraction with 0.5 *M* HCl after washing with water, recentrifugation and counting by earlier procedure[22] showed significant bound radioactivity.

Oxidation of cocaine at ambient temperature with hydrogen peroxide reportedly[23] leads to the formation of fairly stable nitroxide free radical and such radicals in vivo have been reported[24] to be reduced to norcompounds by membrane sulfhydryl groups. The formation of norcocaine as a metabolite of cocaine in rat brain conceivably may play an important role in the systemic toxicity of cocaine. The lack of persistence of cocaine in rat brain for a prolonged period is similar to that observed previously[19,25] with thebaine, an opioid stimulant which also possesses little or no physical dependence liability. In contrast, depressant narcotic analgesic drugs such as morphine[22] and methadone[16,18] with very high tolerance and physical dependence liability, persisted in the rat brain for prolonged periods even after a single s.c. injection. These observations may have some relevance to the absence of tolerance and physical dependence liability with cocaine[26].

Zusammenfassung. Nach s.c. und i.v. Injektionen von Cocain in Ratten wurde Cocain im Hirn rasch metabolisiert. Ausser Benzoylecgonin, Benzoylnorecgonin und Ecgonin wurde Norcocain als wichtiger Metabolit identifiziert.

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Stimulation des tubulären Transportes organischer Basen bei Ratten verschiedenen Alters

Für die renale Ausscheidung von Fremdstoffen wie von körpereigenen Stoffen haben die Carriersysteme für den Transport organischer Säuren und organischer Basen grosse Bedeutung. Diese Carrier haben eine limitierte Transportkapazität, die in der frühen postnatalen Periode geringer ist als im Erwachsenenalter[1]. An Nierenrindenschnitten wurde nachgewiesen, dass durch die wiederholte Gabe von Fremdstoffen eine Aktivierung jenes Carriersystems eintritt, das den betreffenden Fremdstoff transportiert[2,3]. Durch die wiederholte Zufuhr von organischen Säuren kann eine beschleunigte renale Ausscheidung von *p*-Aminohippursäure erreicht werden, die durch den Säurecarrier tubulär sezerniert wird[4].

An jungen und erwachsenen Ratten wurde untersucht, ob durch die wiederholte Zufuhr der organischen Base *Tris* (-hydroxymethyl)aminomethan (Trometamol, THAM) eine beschleunigte renale Ausscheidung dieser Verbindung erreicht werden kann, deren renale Exkretion bei Zufuhr grosser Mengen vorzugsweise durch den Basencarrier erfolgt[5].

Methode. Wistar-Ratten (Jena) verschiedenen Alters erhielten über 4 Tage zweimal täglich um 8 h und um 16 h 94 mg/100 g KG THAM i.p. Am 5. Tag wurde die Geschwindigkeit der renalen Ausscheidung von THAM in einem 3stündigen Diureseversuch im Vergleich zu Kontrollen bestimmt. Die Tiere waren am Tag des Diurese-Versuchs 5, 15, 33, 55, 105 bzw. 240 Tage alt. Kontrollen und vorbehandelte Tiere erhielten zu Beginn des 3stündigen Diureseversuchs 94 mg/100 g KG THAM i.p., wobei die Kontrolltiere im Versuchszeitraum nur etwa die Hälfte der applizierten Menge ausscheiden können. 33 und 55 Tage alte Ratten erhielten die doppelte Dosis, um bei der grösseren Ausscheidungsfähigkeit dieser Tiere im Vergleich zu jungen und alten Ratten eine beschleunigte renale Ausscheidung nachweisen zu können[6]. Das Lösungsmittelvolumen betrug unabhängig von der Dosis 5 ml/100 g KG. Die Bestimmung von THAM erfolgte nach der Methode von ROSEN[7]. Es werden arithmetische Mittelwerte mit Standardfehler angegeben. Die im Versuchszeitraum von 3 h ausgeschiedenen THAM-Mengen wurden auf 100 g KG bezogen. Bei Ratten aller Altersgruppen war die bei der Vorbehandlung applizierte THAM-Menge zu Beginn des Diurese-Versuchs ausgeschieden.

Ergebnisse und Diskussion. Wie die Figur zeigt, führt die wiederholte Gabe von THAM bei Ratten aller Altersgruppen zu einer beschleunigten renalen Ausscheidung dieser Verbindung. Bei 33 und 55 Tage alten Ratten sind die ausgeschiedenen THAM-Mengen grösser als bei den

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