drinking water has no significant effect on either the turnover rate of $^{35}SO_4$ or the initial specific activity (obtained by extrapolation) of kidney or liver. This result is somewhat surprizing in view of the effects on $^{35}SO_4$ incorporation in vitro previously reported for other tissues[8,9]. The possibility that this dose is inadequate can probably be ruled out because it has been shown that 50% larger doses of aspirin are fatal in 3–4 weeks and also that the dose used in these experiments causes morphological changes in the kidney[10]. Thus it must be concluded that sulphate incorporation and turnover in liver and kidney is rather insensitive to aspirin in vivo.

On the other hand aspirin does have a significant effect on ^{14}C-glucosamine turnover. In both the mitochondrial and microsomal fractions of kidney the degradation rate is decreased and the half life increased from 3.92 to 4.94 days and from 3.62 to 5.11 days respectively ($p < 0.05$). In the case of liver there is also a tendency for the half life to be increased and even though the differences are not significant at the 95% level the results do reinforce the data obtained for kidney. In no case, however, is there any significant effect on the initial specific activity. The results suggest that aspirin may be causing a slower replacement of the mucopolysaccharide backbone, without affecting the rate of sulphate replacement. Other workers[11–13] have suggested that aspirin may have a specific effect on amino sugar metabolism, rather than on other stages of mucopolysaccharide biosynthesis.

Table II shows the effect of aspirin on the turnover of ^{14}C-leucine. In this series of experiments there is a tendency for the half life of the leucine to be decreased, as suggested by MENDELSON et al.[5]. However, the variability of the data is higher than in the case of glucosamine and the differences observed are not statistically significant. Nevertheless it is clear that the tendency is in the opposite direction to that observed for glucosamine.

Throughout this work the apparent decay constants are being measured and the possibility that aspirin may be affecting the extent of reutilization rather than the actual degradation rate must be considered. However the most likely way in which reutilization could be affected would be if aspirin modified the rate of loss of small molecular weight degradation products from the cell. If this were the case one would expect all rates to be modified in the same direction and the fact that this is not observed argues against the effect being on reutilization.

One possible hypothesis which would explain the kidney necrosis caused by salicylate containing mixtures is that these mixtures may interfere with membrane formation by inhibiting mucopolysaccharide synthesis. Because kidney function is particularly dependent on membrane integrity and because there is evidence for a mechanism causing salicylate concentration in the kidney medulla[14] this tissue would be expected to be particularly susceptible to this type of damage. The increased half life of glucosamine reported in this paper supports the idea that salicylate ingestion causes a decrease in synthesis and thus provides support for the hypothesis. The fact that sulphate turnover is not affected seems to indicate that its metabolism is less sensitive to salicylate, in keeping with the results of other workers[11–13].

Résumé. L'ingestion d'aspirine (ester acétylsalicylique) entraîne une diminution significative de la vitesse apparente de la dégradation de la ^{14}C-glucosamine dans le rein, sans modifier la vitesse de dégradation du ^{35}S-sulfate; la vitesse de dégradation de la ^{14}C-leucine semble augmenter. Le foie donne les mêmes résultats.

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The Effect of Drugs on the Release of Endogenous Catecholamines into the Perfusate of Discrete Brain Areas of the Cat in vivo

Many psychoactive drugs alter the turnover of brain catecholamines[1]. However, until recently it has not been possible to directly measure the endogenous catecholamines released from cerebral neurons in vivo. Methods have now become available to determine picogram quantities of dopamine (DA) and noradrenaline (NA)[2]. Using these techniques we have investigated the effects of various drugs on the output of DA and NA into the perfusate of discrete brain areas of the cat in vivo.

Methods. Cats of either sex (2.5–3.5 kg) were initially anaesthetized with ether, a catheter inserted into the femoral vein and tracheotomy performed. All wound and pressure points were repeatedly infiltrated with local anaesthetic. After i.v. injection of gallamine the animals were artificially ventilated and ether was withdrawn. The head of the caudate nucleus or the nucleus ventromedialis of the hypothalamus were perfused with warmed physiological Ringer solution (30 µl/min) by means of a push-pull cannula (2 parallel cannulae welded together, outer diameter of each cannula 0.20 mm) implanted stereotaxically (coordinates: caudate nucleus, A = 16, L = 3.8, H = +4.5; hypothalamus, nucleus ventromedialis, A = 11, L = 1.5, H = −4.5, according to the Atlas of SNIDER and NIEMER[3]). The perfusate of the first 30 min was discarded, thereafter 20-minute samples were collected into chilled centrifuge tubes containing 50 µl of 0.01 *N* perchloric acid. The drugs were injected

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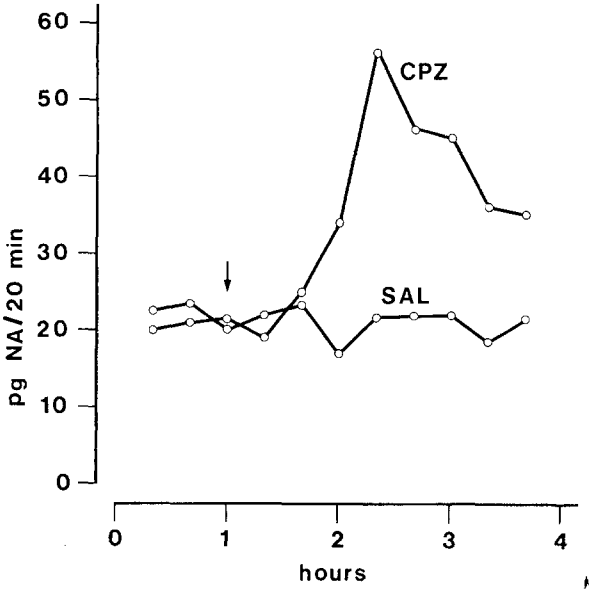
Dopamine content of the perfusate of the head of the cat caudate nucleus

Treatment (mg/kg i.v.)	Postinjection period (min)	Dopamine (pg/20 min) [a]	
Saline	0–60	0.13 ± 0.02	(8)
	60–120	0.15 ± 0.04	(4)
d-amphetamine (10)	0–60	1.08 ± 0.50	(4) [b]
Chlorpromazine (10)	0–60	0.41 ± 0.05	(3) [c]
	60–120	0.58 ± 0.04	(3) [c]
Oxotremorine (0.25)	0–60	1.76 ± 0.19	(3) [c]

[a] For each animal the dopamine content per 20-min sample was averaged over the postinjection period indicated. In parentheses, number of animals. [b] $p < 0.02$; [c] $p < 0.001$, vs saline treatment (Student's t-test).



Typical output of noradrenaline (NA) from the hypothalamus of the gallamine-immobilized cat. Saline (SAL) or chlorpromazine (CPZ) was injected (arrow) 1 h after beginning of the sample collection (= point 0 on the abscissa).

i.v. 1 h after the beginning of the sample collection. The samples were frozen at −80 °C until DA (caudate nucleus) and NA (hypothalamus) were measured by the radioenzymatic method of COYLE and HENRY[2]. Briefly, in the presence of catechol-o-methyltransferase (^{3}H-methyl)-S-adenosylmethionine (SA: 5 Ci/mmole, Amersham, GB), DA and NA were converted into their labelled methylated derivates which were then extracted into organic solvents. Standards of DA and NA (0.02–1 ng) added either to Ringer solution or to samples of perfusate yielded similar amounts of radioactivity which were linear within the concentration range indicated.

Results. 1. The release of DA within the caudate nucleus was constant during at least 2 h following i.v. injection of saline (Table). 2. Administration of D-amphetamine (10 mg/kg, i.v.) increased the output of DA from the caudate nucleus about 8-fold as compared to the preinjection (control) period (Table). 3. The neuroleptic drug chlorpromazine (10 mg/kg, i.v.) enhanced the output of both DA from the caudate nucleus (Table) and NA from the hypothalamus (Figure) during at least 2 h. 4. Oxotremorine (0.25 mg/kg, i.v.) resulted in a large increase in the DA content of the perfusate of the caudate nucleus (Table).

Discussion. Liberation of DA from the striatum has been previously shown. Radioactive DA or water formed from labelled tyrosine was collected by superfusing the caudate nucleus by means of the cup method[4]. Moreover, endogenous DA[5], as well as labelled DA accumulated in the striatum following intraventricular injection of the amine[6], were recovered in the ventricular perfusate after electrical stimulation of the substantia nigra. However, labelled tyrosine and/or DA may not uniformly mix in the endogenous pools and the ventricular DA may originate from structures other than the striatum (e.g. nucleus accumbens septi). We have measured the minute amounts of the endogenous DA released within the cat caudate nucleus using a sensitive radioenzymatic method. An average basal amount of 130 pg DA per 20 min has been found. These results considerably differ from those previously reported (10,000 pg/10 min) using a similar perfusion technique but with a perfusion rate of 120 µl/min and fluorimetric determination of DA[7]. However, our values are of a similar order of magnitude as those obtained by superfusing the caudate nucleus[8].

The increased release of DA in the striatum following chlorpromazine together with the virtually unchanged concentration of the amine in the perfused tissue (unpublished) provides direct evidence that the drug enhances the turnover and the synaptic release of DA. This effect is thought to be compensatory to the impairment of dopaminergic transmission probably as a consequence of the blockade of DA receptors[9].

Accordingly, promethazine, a phenothiazine derivative devoid of DA-receptor blocking properties, failed to enhance the DA output from the caudate nucleus (in preparation). In addition, chlorpromazine, due to its α-adrenergic blocking properties[10], enhanced the NA-release from the hypothalamus, probably as a result of a feed-back activation of noradrenergic neurons.

An enhanced release of striatal DA was caused also by D-amphetamine and oxotremorine. However, the action mechanisms of these compounds are different from that of neuroleptic drugs. Thus, D-amphetamine has been proposed to liberate the amine by a direct action on its storage sites[11]. In contrast, the enhancement of DA release caused by oxotremorine is probably due to stimulation of excitatory cholinoceptive sites, possibly located on the dopaminergic nigro-striatal neurons[12,13]. In preliminary experiments this action of oxotremorine

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was blocked by atropine but not by methylatropine thus excluding involvement of extracerebral mechanisms on the striatal DA release. The view that DA neurons receive an excitatory cholinergic influence is further supported by the findings that anti-acetylcholine agents decrease the DA turnover and counteract the enhanced liberation of the amine caused by neuroleptic drugs[14,15]. Conversely, cholinergic neurons in the striatum receive an inhibitory dopaminergic input: in fact, impairment of DA transmission by neuroleptic drugs results in an increased output of acetylcholine into the perfusate of the cat caudate nucleus and this effect is prevented or reversed by dopaminergic agents such as L-dopa or apomorphine[16].

The results obtained with oxotremorine together with the findings on acetylcholine release indicate a functional interconnection between cholinergic and dopaminergic neurons in the extrapyramidal system, which might mediate rapid feed-back mechanisms modulating the activity of these neurons[16]. For instance, the enhanced DA turnover caused by neuroleptic drugs may result not only from blockade of presynaptic DA receptors[17] but also from disinhibition of a cholinergic system which activates the dopaminergic neurons.

In conclusion, the present results show that the effect of drugs on the release of pg quantities of endogenous DA and NA from discrete brain areas can be directly investigated using the push-pull cannula perfusion technique. The output of catecholamines from various brain regions can also be measured in the unrestrained unanaesthetized cat by means of chronically implanted push-pull cannulae (in preparation), as previously described for acetylcholine release[18].

Zusammenfassung. Hirnregionen der gallamin-immobilisierten Katze wurde mittels «push-pull»-Kanülen perfundiert. Die im Perfusat freigesetzten endogenen Katecholamine wurden radioenzymatisch gemessen. Chlorpromazin, D-Amphetamin oder Oxotremorin (i.v.) erhöhten den «output» von Dopamin aus dem Nucleus caudatus und Chlorpromazin zusätzlich denjenigen von hypothalamischem Noradrenalin durch verschiedene Mechanismen.

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Increase in Food Consumption and Growth after Treatment with Aminoguanidine

In an earlier study, we observed an increase in appetite in a patient with medullary carcinoma of the thyroid upon treatment with the diamine oxidase (DAO, histaminase) inhibitor, aminoguanidine[1]. DAO is produced in large amounts by this tumor[1-4], and aminoguanidine was administered to see if inhibition of DAO influenced tumor growth. During treatment, the patient noted an increase in her appetite, she ate well, her weight increased, although no regression of tumor was noted[1]. On the basis of this observation, further studies were undertaken in rats to see if aminoguanidine had a specific effect on appetite. In addition, the distribution of this drug and its effect on diamine oxidase activity in various tissues was studied.

Methods and materials. The patients included 2 subjects with widely disseminated medullary carcinoma of the thyroid who were chronically ill. The first was a 19-year-old white female. She received the cytotoxic agents, Cytoxan and Vincristine. She was subsequently treated with aminoguanidine, 30 mg/day (3 × 10 mg), for a period of 5 months and then for 2 months. Therapy was discontinued 2 weeks before her death. The second patient, a 45-year-old male, was admitted to NIH in an almost moribund condition. He received aminoguanidine, 30 mg daily, but died 17 days after admission to NIH while still on the drug. The history of these patients has been described in detail elsewhere as Patient 1 and 8[2].

For the animal studies, male Sprague-Dawley rats (Zivic Miller, Inc., Allison Park, Pa., average weight, 80 g) were housed, individually, in metal cages and were given water and powdered Purina Chow ad libitum. Food was supplied in stainless steel dishes with concave lids having an aperture of 3.4 cm diameter to prevent spillage. Room lighting (12 h on, 12 h off) and temperature (23–25 °C) were controlled. Aminoguanidine sulfate was administered orally by stomach tube in doses of 10 or 50 mg/kg daily in distilled water. Control animals received water alone. Measurement of food consumption and body weight were made daily.

At the end of each experiment, the animals were killed by decapitation. Tissues were rinsed briefly in water, blotted and frozen on dry ice for storage at −20 °C. Aminoguanidine was assayed by reaction with *p*-nitrobenzaldehyde as described by BEAVEN et al.[5]. Diamine oxidase activity was measured by the procedure of BEAVEN and JACOBSEN[6]. This procedure measures the release of tritiated water from side chain labeled β-^{3}H-histamine upon deamination.

Aminoguanidine sulfate and *p*-nitrobenzaldehyde were purchased from Eastman Kodak Company, Rochester, N.Y.; β-^{3}H-histamine was prepared from β-^{3}H-L-histidine as described previously[6].

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