The presence of dopamine in cat spleen and blood

We have measured the resting concentrations of noradrenaline and dopamine in the spleen and blood of cats under chloralose anaesthesia. Using a modification of the column separation method of Bertler, Carlsson & Rosengren (1958), and the very sensitive assay methods of Vendsalu (1960) for noradrenaline and adrenaline and of Laverty & Sharman (1965) for dopamine, and expressing the catecholamine content of the spleen in terms of its tissue mass as estimated by its content of deoxyribonucleic acid phosphorus (Dearnaley & Geffen, 1966), we have made the following observations.

From 22 cats the mean resting concentration of dopamine in the spleen was found to be 4·82 ng/μmol DNA-P (s.e. $\pm$ 1·16) which was approximately 10% of the noradrenaline content (mean 50·20 $\pm$ 5·71 ng/μmol DNA-P, n = 16). In 9 other cats, however, the dopamine content was estimated to be some 4 to 20 times higher than this (mean 33·77 $\pm$ 8·55 ng/μmol DNA-P), and a few spleens therefore contained more dopamine than noradrenaline. Why these spleens contained so much more dopamine than the others we do not know (there appeared to be no obvious correlation between factors such as age, sex or diet and the high dopamine levels) but we feel sure that it was dopamine for a number of reasons.

We have been able to show, for example, that dopamine is present in plasma from the blood of some cats at a resting concentration of 0·69 to 1·52 ng/ml, but in the plasma from other cats we found levels much in excess of this (3·85 to 14·46 ng/ml). These levels do not take into account the low recovery of dopamine, 25%, which was obtainable from plasma by the techniques adopted. In those animals where both blood and spleen concentrations of dopamine were measured they were both in either the low group or the high group. Similarly, in some of our experiments where cat spleens have been divided (Dearnaley & Geffen, 1966), when one portion was shown to contain high levels of dopamine, perfusion of the other portion with McEwen solution (Thoenen, Hürlimann & Haefely, 1963) resulted in the appearance of large amounts of dopamine in the effluent (1·49 to 4·26 ng/ml after 30 min perfusion). This was in contrast to the situation in spleens containing low levels of dopamine (less than 0·08 ng/ml). Finally we have shown that the fluorescence characteristics of the amine from spleens apparently containing high levels of dopamine corresponds to that of authentic dopamine, and that the fluorescence intensity and hence the dopamine content of samples was essentially the same before and after subjecting the samples to chromatography and elution at the dopamine Rf value (Laverty & Sharman, 1965).

The experiments in which the spleens were perfused, also allow us to speculate on the nature of the storage of this dopamine in the tissue. When the wash-out of dopamine from spleens with a high dopamine content was determined at various times during the perfusion, the rate of output declined in an exponential manner (Fig. 1) similar to the decline observed by Iversen (1965) after the accumulation of adrenaline and noradrenaline by the Uptake$_2$ process in the rat isolated heart. We can demonstrate an identical wash-out of accumulated dopamine after its infusion into spleens containing dopamine at the lower level and suggest therefore that the high levels of dopamine found in some of our experiments had accumulated in Uptake$_2$ tissue storage sites. Whether the high spleen levels precede the high blood levels or vice versa we do not know, nor is the original source of this dopamine apparent. We hestitate to comment on the possible functional significance of our findings but it would appear that endogenous amines can occur in Uptake$_2$ storage sites.

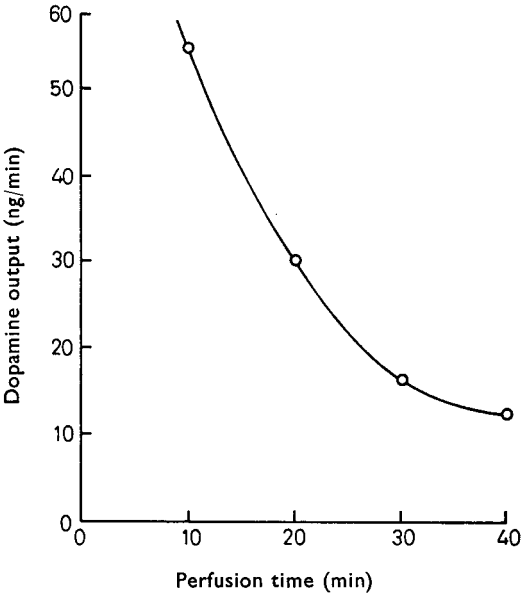

FIG. 1. The relation between the rate of output of dopamine (ng/min) and the time of perfusion of a cat isolated spleen containing a large amount of dopamine.

Table 1. *The influence of pargyline hydrochloride on catecholamine levels in cat spleen and plasma*

		Spleen content (ng/μmole DNA-P)		Plasma concentration (ng/ml)	
		Untreated (n = 16)	Pargyline pretreated (n = 15)	Untreated (n = 6)	Pargyline pretreated (n = 7)
Noradrenaline	Mean	50·20	30·09	1·15	1·34
	s.e.	±5·71	±4·88 (P <0·01)	±0·17	±0·24
Adrenaline	Mean	14·68	7·72	1·66	0·60
	s.e.	±2·10	±1·92 (P <0·001)	±0·44	±0·15 (P <0·05)
Dopamine	Mean	7·66	19·16	4·75	4·40
	s.e.	±2·16	±4·38	±2·15	±1·27

Pargyline (50 mg/kg) was administered subcutaneously 16 h before the experiment.

Pretreatment of cats with the monoamine oxidase inhibitor pargyline, 50 mg/kg injected subcutaneously 16 h before the experiment, was found to significantly lower the noradrenaline and adrenaline contents of spleens and the adrenaline content of plasma. The dopamine content of both spleen and plasma was not significantly altered by pargyline (Table 1).

Department of Pharmacology,
Portsmouth School of Pharmacy,
Park Road, Portsmouth, Hants, England.

DENISE M. STREET
D. J. ROBERTS

January 8, 1969

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Removal of plasma metyrapone in rats submitted to previous pharmacological treatment

Metyrapone [SU 4885; Metopirone; 2-methyl-1,2-di(3-pyridyl)propan-1-one] a relatively specific 11-β-hydroxylase inhibitor, is currently used for testing the pituitary ACTH reserve (Liddle, Estep & others, 1959). Several authors have reported that the metyrapone test is not reliable in patients under therapy with other drugs like for instance phenobarbitone and diphenylhydantoin (Krieger, 1962; Rinne, 1966, 1967; Werk, Thrasher & others, 1967). Metyrapone is metabolized by the liver to form a reduced compound [SU 5236; 2-methyl-1,2-di(3-pyridyl)propanol] (Kraulis, Traikov & others, 1968), a process which is blocked *in vivo* by the administration of an inhibitor of liver microsomal enzymes such as SKF 525 A (S. Szeberenyi, unpublished). It may be possible therefore that the level and the disappearance of metyrapone from plasma are affected by treatment with various drugs known to influence the activity of liver microsomal enzymes. This note summarizes preliminary data obtained by measuring the half-life of metyrapone in plasma of rats pretreated with several drugs known to increase the rate of metabolism (induction) of foreign compounds. Female Sprague–Dawley rats (140 g) were treated with various drugs twice a day (9.00 a.m. and 9.00 p.m.) for 5 days as reported in Table 1. 36 h after the last treatment, metyrapone hydrochloride was injected intraperitoneally at the dose of 66 mg/kg. 5, 15 and 30 min after metyrapone administration, animals were killed and metyrapone was determined in plasma according to the method of Szeberenyi, Szalay & Tacconi (1968). At least 12 animals per drug were used.

Table 1. *Half-life* ($t\frac{1}{2}$) *of metyrapone in plasma of rats pretreated with several drugs*

Drug	mg/kg (twice daily for 5 days)	$t\frac{1}{2}$ (min) of metyrapone in plasma
Saline	(0·5 ml)	17 ± 0·6
Niketamide	100 orally	14
Phenylbutazone	62·5 i.p.	13·5
Diazepam	50 i.p.	13
Meprobamate	100 i.p.	12
Diphenylhydantoin	37·5 i.p.	12
Pentaerythritol tetranitrate	25 i.p.	11
Hydrocortisone	40 s.c.	10·5
Phenobarbitone	37·5 i.p.	9