administered with equal ejecting currents. The effect of this substance was not antagonized by strychnine administered electrophoretically in concentrations which blocked the depressant action of glycine.[3] Thus, with respect to its central action, muscimol is apparently a "GABA-like" amino acid,[3] as anticipated on structural grounds.[4]

It remains to be established whether ibotenic acid or muscimol pass through the blood–brain barrier after ingestion, so contributing to the neurological manifestations of *Amanita* poisoning. The excitant action of ibotenic acid and the depressant action of muscimol is in accordance with previously established structure–activity relationships for amino acids,[4] α-decarboxylation of an excitant leading to an amino acid with depressant activity. However, these isoxazoles are the first heterocyclic amino acids exhibiting such activity, and their relatively strong effects on central neurones indicates that further examination of analogues of glutamic acid and GABA which are of similarly restricted conformation might be rewarding in understanding the interaction between amino acids and membrane receptors.

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Intracellular distribution of pyridine nucleotides in the liver of rats after long-term exposure to carbon disulphide*

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PROLONGED exposure to carbon disulphide leads to disturbances in the urinary excretion of nicotinamide metabolites both in animals[1–3] and in human beings.[4] It seemed interesting to investigate whether the above phenomena are accompanied by changes in the levels of the individual pyridine nucleotides in some of subcellular fractions of liver cells.

The levels of the individual pyridine nucleotides were recently investigated in connection with exposure to other chemicals and some changes were found after administration of substances showing hepatotoxic and carcinogenic properties.[6–10]

METHODS

Female albino rats of the Wistar strain were exposed in a toxicological chamber to CS_2 vapours at concentration of approximately 2 mg/l. of air, for 5 hr daily, 6 days in the week for the total of 9

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months. After this period all rats in the experimental group displayed typical symptoms of chronic carbon disulphide intoxication, such as loss of motorial equilibrium and muscular weakness or even paraplegia of the posterior limbs. The control animals were kept over the same time period without exposure. When sacrificed for the determination of the pyridine nucleotides all rats were about 12 months old.

The animals were sacrificed in pairs: one rat after CS_2 exposure and a control one. The liver was removed and it was immediately homogenized in 0·25 M sucrose containing 10 mM Tris-HCl buffer, pH 7·2, 10^{-4} M EDTA and 0·05 M nicotinamide. The procedure was that of Schneider,[11] slightly modified: homogenization was carried out for 2 min in a glass homogenizer with plexiglas piston, the rotation rates being approximately 600/min. Debris and nuclear fraction was centrifuged at 600 *g* for 10 min, mitochondria at 3300 *g* for 10 min, microsomes at 104,000 *g* for 30 min. The nuclear and mitochondrial fractions were washed once with 0·25 M sucrose; for the microsomal fraction this step was omitted. The total time of fractionation was about 2·5 hr. All centrifuging was carried out in "Heinz Janetzki" centrifuges, model K-14/A and VAC-60, at 0–2°C.

The total protein in each fraction was determined according to Lowry *et al.*[12] with bovine serum albumine as a standard. The activity of the succinic dehydrogenase (reference enzyme for the mitochondrial fraction) was determined spectrophotometrically using the method of Bonner.[13]

Pyridine nucleotides concentration in the whole homogenate and in the individual cellular fractions were determined using a fluorometric method elaborated previously in this laboratory for the whole liver tissue.[14] Oxidized nucleotides (NAD, NADP) were extracted with cold 5% trichloroacetic acid. Reduced nucleotides ($NADH_2$, $NADPH_2$) were extracted with hot 0·1 M sodium carbonate and subsequently oxidized quantitatively with phenazine methosulphate. Extraction of nucleotides was carried out immediately after the homogenate or individual cellular fraction was obtained. The fluorescence was developed by incubation of the sample with 6N NaOH. The developed fluorescence was measured using "Opton" spectrofluorimeter type ZFM4C (exciting light at 370 nm, excited fluorescence at 460 nm, Fig. 1).

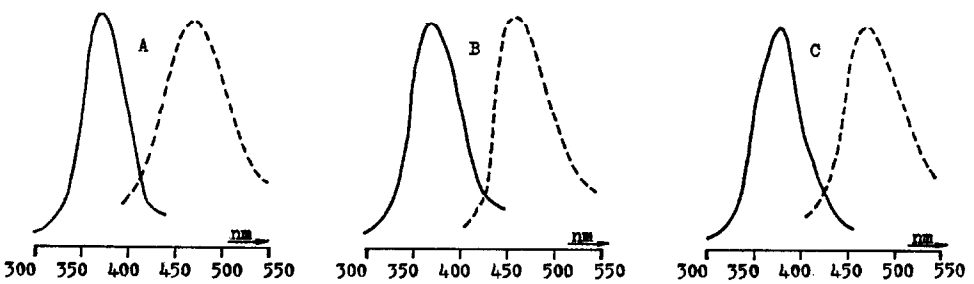

FIG. 1. ——, Light exciting spectrum (maximal transmission at 460 nm); – – – –, spectrum of excited fluorescence (maximal exciting at 370 nm). A-NAD standard solution, B—pyridine nucleotides extracted from rat liver homogenate, C—pyridine nucleotides extracted from whole liver (Incubation: 0·3 ml + 0·6 ml 9N NaOH, 60 min 38° + 3·1 ml H_2O).

RESULTS AND DISCUSSION

The results of investigation are presented in Table 1. There was a 13 per cent decrease in the level of reduced nucleotides in the whole liver homogenate of the experimental group as compared with the control, dependent mainly upon the decrease of these nucleotides in the mitochondria. Some decrease of reduced nucleotides was found also in nuclear fraction. As it may be seen from the distribution of activity of succinic dehydrogenase, this decrease may be attributed to the mitochondrial contamination of the nuclear fraction. Owing to a simultaneous slight elevation of the reduced nucleotides in the soluble fraction a substantial decrease was observed in the mitochondrial : cytoplasmatic ratio of the reduced nucleotides levels amounting to *ca.* 30 per cent of the control (1·28 ± 0·13 for control and 0·92 ± 0·14 for poisoned rats). There was no distinct difference in intracellular distribution of oxidized nucleotides between the control and CS_2 poisoned group, although there was a tendency for the mitochondrial and nuclear level to be depressed.

TABLE 1. INTRACELLULAR DISTRIBUTION OF PYRIDINE NUCLEOTIDES IN THE LIVER OF RATS EXPOSED TO CS_2

Cellular fraction	Group	N	Total protein mg ± S.D.	Succinic dehydrogenase (activity per cent)	NAD + NADP mμM ± S.D.	$NADH_2$ + $NADPH_2$ mμM ± S.D.
Homogenate	CS_2	7	201 ± 9	100	623 ± 26	456 ± 62†
	control	6	204 ± 8		629 ± 62	523 ± 27
Nuclear fraction and debris	CS_2	7	36 ± 5	18	43 ± 7	50 ± 15*
	control	6	34 ± 8		49 ± 7	67 ± 15
Mitochondrial fraction	CS_2	7	48 ± 3	73	115 ± 18	203 ± 37‡
	control	6	48 ± 3		124 ± 20	254 ± 24
Microsomal fraction	CS_2	7	33 ± 3	9	49 ± 4	29 ± 4
	control	6	31 ± 3		49 ± 11	27 ± 8
Soluble fraction	CS_2	7	87 ± 7	0	410 ± 17	221 ± 25
	control	6	88 ± 7		415 ± 54	199 ± 18

The levels of proteins and nucleotides are expressed per 1 g of wet liver tissue.
Difference statistically significant in relation to control: * $0{\cdot}05 < P < 0{\cdot}1$; † $0{\cdot}02 < P < 0{\cdot}05$; ‡ $0{\cdot}01 < P < 0{\cdot}02$.

The ratio (NAD + NADP)/($NADH_2$ + $NADPH_2$) was altered in another direction in the cytoplasm (decrease) and mitochondria (increase) of intoxicated rats, however, these differences are statistically insignificant and interpretation is difficult.

The present results indicate that in the chronic CS_2 intoxication there are only slight changes in the levels of the pyridine nucleotides in the liver; not so profound for instance as those which were produced by high doses of CCl_4.[7] Nevertheless, taking into account lowering of the level of the mitochondrial nucleotides and the property of CS_2 to act as an inhibitor of cytochrome oxidase[15] it may be assumed that this toxic agent may influence the pyridine nucleotides-linked processes in mitochondria.

The level of pyridine nucleotides in subcellular particles of liver cells in normal rats found in this investigation was distinctly higher as compared with the data based on the similar fractionation technique reported previously by other authors (Table 2). It has been recently proved by Slater[16, 17]

TABLE 2. PYRIDINE NUCLEOTIDES LEVEL IN SUBCELLULAR STRUCTURES (NUCLEI + MITOCHONDRIA + MICROSOMES) AND IN THE SOLUBLE CYTOPLASMIC FRACTION OF THE RAT LIVER

Nucleotides	Author	Homogenate (mμM)	Homogenate (%)	Cellular structures (mμM)	Cellular structures (%)	Soluble fraction (mμM)	Soluble fraction (%)
NAD + NADP	Jacobson and Kaplan[19]	728	100	93	13	519	71
	Glock and McLean[18]	671	100	144	21	573	85
	Authors' data	629	100	222	35	415	66
$NADH_2$	Jacobson and Kaplan[19]	446	100	124	28	129	29
+	Glock and McLean[18]	486	100	203	42	194	40
$NADPH_2$	Slater[17]* (a)		100		27		38
	(b)		100		56(49)		51(58)
	Authors' data	523	100	348	67	199	38

The levels of nucleotides are expressed per 1 g of wet liver tissue.

* Data for $NADPH_2$, (a) results obtained using the classical method of differential centrifuging, (b) results obtained using the so called rapid fractionation (in parenthesis are given approximate data for reduced nucleotides, calculated from the relation $NADH_2/NADPH_2$ according to Glock and McLean[18]).

that the level of pyridine nucleotides in the particles had been found higher when rapid separation of cellular fractions was used as compared to those obtained by means of the standard technique of differential centrifuging. This finding may suggest that there is a substantial loss of nucleotides contained originally in the particles due to which the level in the particles has a tendency to be lowered with the duration of fractionation procedure. The present investigation was based on the classic fractionation method, however, the level of pyridine nucleotides found in subcellular particles was very high in relation to the whole homogenate and amounted to 67 per cent for the reduced and 35 per cent for the oxidized nucleotides, respectively. At the present stage it would be difficult to decide whether these discrepancies should be attributed to differences in the methods of determination of pyridine nucleotides and in fractionation technique or to the differences in the biological material used by various authors.

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Hyposensitivity to 5-hydroxytryptamine in the isolated stomach fundus of the newborn rat—II. Amphetamine and receptor deficit

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We have previously shown the occurrence of a reduced affinity (by a factor of −23) for 5-hydroxytryptamine (5-HT) in the isolated gastric fundus of the newborn rat.[1] Intrinsic minor contractility of the smooth muscle not yet fully developed cannot be entirely accepted as only reason of the hyposensitivity. Other more specific reasons are possible: among them a 5-HT receptor *deficit*, in the newborn animal. This hypothesis is examined in the present paper.

The investigation is based upon the assumption that amphetamine acts directly on the 5-HT receptor[2–4] in several isolated organ preparations, among them rat fundal strips.[3] Assuming that the concept of identical receptors for the two amines is correct, one would predict neonatal hyposensitivity also to amphetamine. This reduced affinity should be similar to that evidenced with 5-HT, and might support the 5-HT receptor deficit hypothesis. Both DL- and D-amphetamine are tested in the present experiments, to control possible differences between the racemic drug and the optical isomer with higher direct action on the 5-HT receptor.[3]

MATERIALS AND METHODS

Ninety-five Wistar albino rats of either sex were used. The gastric fundus preparations were described in detail in a previous paper.[1] Cumulative log dose–response curves were obtained with the drugs. Significance was tested on the pD_2 (affinity[6]) mean values, by Fisher's-F-test.[7]

The following drugs were employed: 5-hydroxytryptamine creatinine sulphate (Merck, Germany) D-amphetamine sulphate (Merck) and DL-amphetamine sulphate (Merck). Doses are expressed as base.