messenger RNA is formed which acts on ribosomes to stimulate specific enzyme production. Ui and Mueller[5] have reported that actinomycin D blocked RNA synthesis in the rat uterus and also blocked the action of the hormone estradiol on the uterus. They concluded that the action of estradiol depended upon the synthesis of new RNA and, consequently, a stimulation of protein synthetic mechanisms.

TABLE 1. EFFECT OF ACTINOMYCIN D ON ALDOSTERONE-INDUCED CHANGES IN THE EXCRETION OF ELECTROLYTES IN ADRENALECTOMIZED RATS

Treatment	Total excretion in 4 hr, mEq Sodium	Potasssium
None	0·41 |	0·08 |
Actinomycin D	0·44	0·13
Actinomycin D and aldosterone	0·36 |	0·22 |
Aldosterone	0·13	0·23
Coefficient of variability	42%	25%

* Values for each treatment represent the means of four animals. In each column, any two means joined by a vertical line are not significantly different. Any two means not so joined are significantly different (5% level). Statistical analysis was performed by analysis of variance using a completely randomized block design and Duncan's new multiple range test.[3]

The blockade by actinomycin D of the action of aldosterone on sodium excretion presented here could be interpreted to occur by the mechanism proposed by Karlson.[4] The delay in onset of action of mineralocorticoids would thus be due to the time necessary to initiate synthesis of a protein involved in the transport of sodium. The lack of any effect of actinomycin D on aldosterone-induced kaluresis would indicate that this action can be separated from the antinatriuretic response and hence is mediated in another manner.

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Localization of acetylcholine, 5-hydroxytryptamine and noradrenaline within subcellular particles derived from guinea pig subcortical brain tissue

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WHEN brain tissue is homogenized in 0·32 M sucrose, nerve endings escape disruption and are snapped off to form nerve-ending particles (NEPs) which can be isolated in a discrete fraction by differential

and density gradient centrifugation.[1-3] On suspension in water, the outer membranes of the NEPs are disrupted, and soluble cytoplasmic constituents together with some synaptic vesicles are released. A simple density gradient procedure[4,5] permits the separation of fractions: fraction *O* containing soluble cytoplasm; *D*, synaptic vesicles; *E*, microsomes; *F* and *G*, membrane fragments and NEP ghosts; *G* and *H*, partially ruptured NEPs and *I*, mitochondria. Bound acetylcholine was found to be bimodally distributed with peaks in the synaptic vesicle (*D*) and damaged NEP (*H*) fractions.

Other work[6-8] has shown that 5-hydroxytryptamine (HT) and noradrenaline (NA) are also localized in the NEP fraction (although not so sharply as acetylcholine). The object of the present work was to find out whether these two amines behaved similarly to acetylcholine on hypotonic disruption and in particular whether a synaptic vesicle fraction containing them could be isolated.

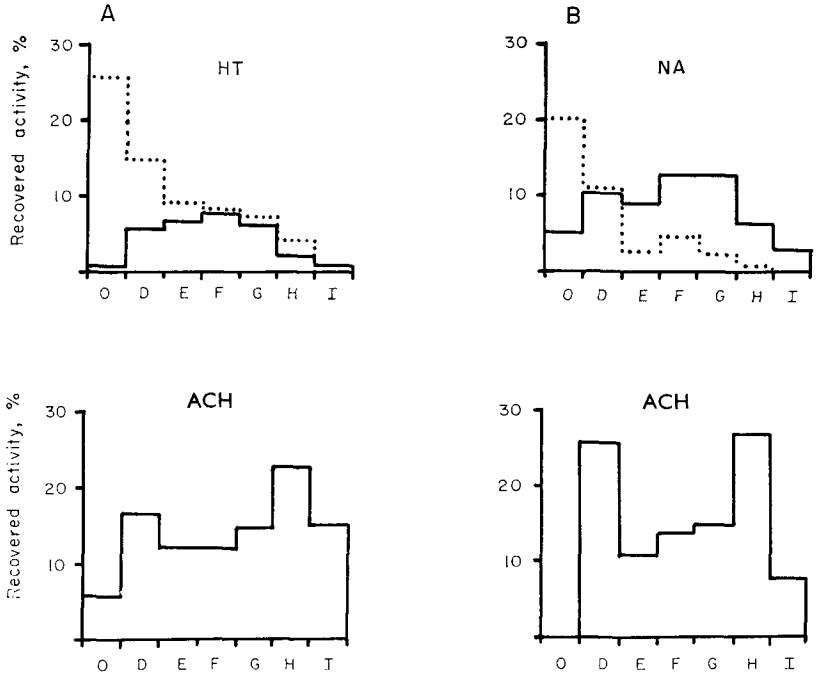

FIG. 1. Comparison of the distribution within a density gradient of (A) 5-hydroxytryptamine (HT) and acetylcholine (ACh), and, (B) noradrenaline (NA) and acetylcholine (ACh) derived from hypotonically-treated subcellular particles from guinea pig subcortical brain tissue. Solid lines represent bound amine, dotted line represents free amine. Ordinate: percentage in each fraction of the total amine in the gradient. Amine recovered, as mμmole/g tissue processed (no. of experiments averaged and percentage recovery in brackets): HT, 1·27 (3, 137); ACh, 0·82 (2, 66); NA, 1·1 (2, 123); ACh, 5·8 (3, 132).

Some technical difficulties were encountered. The estimation of HT as total 5-OR indolyl compounds without solvent extraction as used earlier[4] gave falsely high values with fractions from the lower part of the density gradient owing to the presence of interfering substances in the sucrose. This was overcome by solvent extraction into butanol.[9] The levels of the amines, particularly of HT, in whole brain preparations were low. The amines liberated as the free form on hypotonic rupture are not destroyed, as with acetylcholine, so that means had to be devised for separately estimating free and bound amines in each fraction. Accordingly our earlier procedure was modified and two kinds of preparation were used.

In the experiments with HT, the level of the amines was raised by injecting iproniazid (100 mg/kg) subcutaneously 14–20 hr before killing the animals. This procedure does not alter the amine distribution in the primary fractions.[6] The midbrain, diencephalon and caudate nuclei, the regions richest in HT, were homogenized. After removal of the nuclear (P_1) fraction at 1000 *g* for 11 min, a crude

mitochondrial (P_2) fraction was prepared by centrifuging the homogenate at 17000 *g* for 60 min. The P_2 fraction was suspended in water and separated into fractions *O–I* as previously described.[4] Each fraction was diluted with water and centrifuged at 100,000 *g* for 60 min to give supernatants and pellets. The amine present in the supernatants is regarded as "free" and that in the pellets as "bound". All the acetylcholine, as estimated by the leech muscle micromethod[10] after release by heating at 100° for 10 min at pH 4,[11] was recovered in the pellets, i.e. was "bound", since in the absence of an anticholinesterase any free acetylcholine would be destroyed.

In the NA experiments, there was no premedication; only the midbrain and diencephalon were used, since the caudate nucleus is known to contain very little of this amine. The NA was estimated fluorimetrically.[12] Distribution studies with the primary fractions from these tissues showed that about 30 per cent of the NA was recovered in the microsomal (P_3) fraction; accordingly, after separation of the P_1 fraction, a combined P_2 and P_3 fraction was prepared by centrifuging at 100,000 *g* instead of 17,000 *g* for 1 hr. Disruption in water was carried out more vigorously than in the HT series, with mechanical homogenization for about 30 sec. Morphological examination showed that fractions *D–F* were less homogeneous than the corresponding fractions from whole brain; they all contained synaptic vesicles heavily contaminated with microsomes.

The results of the two series of experiments are shown in Fig. 1. In the HT series (Fig. 1A) bound acetylcholine was bimodally distributed with peaks in *D* and *H*. The *D* fraction was less opaque and the yield of vesicular acetylcholine was lower than in previous whole brain studies.[4] (Dog caudate nucleus preparations have also been found to be more resistant to hypotonic rupture than guinea-pig whole brain.) In contrast to acetylcholine, bound HT peaked in *F*, but was not clearly localized in any fraction and showed no evidence of bimodal distribution. About 70 per cent of the total HT recovered was present in the free form; fraction *O*, corresponding to soluble cytoplasm, contained the most, with decreasing amounts in the lower parts of the gradient. The free amine in the lower fractions could have occurred as the result of diffusion from *O*.

In the NA series (Fig. 1B), acetylcholine was again bimodally distributed, and perhaps because of the more vigorous disruption, the opacity of the *D* fraction and the yield of acetylcholine in this fraction was greater. A greater proportion of NA was present in the bound form than was found with HT, but the distribution of bound NA was similar to that of bound HT. The distribution of free NA was also similar to that of HT, the highest concentration being in *O* with decreasing amounts in lower fractions.

To summarize: when particulate fractions containing NEPs are disrupted by exposure to hypotonic conditions, a considerable proportion of free amine is liberated and is recovered in the soluble cytoplasmic (*O*) fraction, with decreasing amounts in lower fractions. In the case of NA, over half the amine remains bound, a proportion similar to that found with acetylcholine[11] and of this significant amounts are associated with particulate material free from NEPs. With HT, the proportion remaining bound is lower and the absolute amounts present are too low to permit firm conclusions as to the nature of the particulate binding sites.

It is obvious from these experiments that the storage sites for NA and HT within the subcellular particles differ from that for acetylcholine and require further characterization. However, it is not yet possible to distinguish between the localization of NA and HT.

In comparing the behaviour of NA and HT, the differences in the preparations used, the vigour with which they were disrupted and a possible selective effect of iproniazid in the localization of HT within the intact storage particle must be borne in mind. Further work is in progress to define more accurately the nature of the amine storage sites.

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Preferential distribution of diphenylhydantoin in primary human brain tumors

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ALTHOUGH the distribution of diphenylhydantoin has been studied extensively,[1-3] little is known of its distribution in human primary brain tumors. In this study of a series of eight patients, seven had astrocytomas of grades III and IV and one a meningioma. Samples of primary brain tumors and adjacent areas of normal brain tissue were obtained from these patients during surgery and kept at −4° until analyzed. Histological identification of the tumor type and normal tissue was made by one

TABLE 1. DISTRIBUTION OF DIPHENYLHYDANTOIN IN HUMAN PRIMARY BRAIN TUMORS AND ADJACENT NORMAL BRAIN TISSUES

Patient	Diagnosis	Dose (mg/day) Days pre-op 1	2	3	4	Concentration (μg/g)* Tumor	Normal	Tumor/ normal
1	Grade IV	100	100			1·156	0·659	1·754
2	Grade IV	200	200	400	200	1·080	0·610	1·770
3	Grade IV	100	400			0·592	0·318	1·862
4	Grade IV	100	100	100		2·724	1·377	1·978
5	Grade III	100	200	200		0·652	0·414	1·575
6	Grade III	100	500	200		1·760	1·620	1·086
7	Grade III	400	400	400		2·173	0·714	3·043
8	Meningioma	800				2·848	0·718	3·967
Mean ±						1·623	0·804	2·129
S.E.						0·31	0·16	0·33

0·005 ml/g as estimated by the method of King and Gilchrist.[4] Analysis of diphenylhydantoin was made by the method of Dill *et al.*[1] Average recovery of drug added to normal brain tissue in a concentration of 2·5 μg/g was 86·2 per cent, with a standard error of 3·1 per cent. The tissue blank value for normal brain tissue was approximately 0·069 μg/g.

of us (A.A.S.). The average blood content of brain tissue after washing with saline was approximately