

tions of [^3H]-proline (40–60 μCi in 0.4 μl) into the medial septal area. α -Butx binding sites were located by incubating fresh cryostat-sections of the rat hippocampus in 10^{-9}M ^{125}I - α -Butx for 20 min followed by washing and standard autoradiographic preparation. Over 60 rat brains taken from animals whose postnatal age ranged from 1 h to more than 60 days were used in this study.

Comparison of the α -Butx binding pattern with cholinergic axon terminal distribution revealed a fairly close correspondence within the stratum oriens of field CA1 and within the hilus of the dentate gyrus. However there was an intense patch of toxin labelling within an area at the mouth of the dentate gyrus in-

cluding a portion of field CA3 which was devoid of both cholinergic termination and associated AChE activity. During development an intense patch of α -Butx binding sites was also seen over the pyramidal cell bodies and the adjacent stratum radiatum of field CA1, but this disappeared after the second postnatal week. This area is not thought to be a site of cholinergic termination in either the adult or developing rat brain.

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The binding of choline acetyltransferase to membrane; metabolism of choline in rabbit cortical slices

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The binding of net positively charged (pI, 8.5) rat brain choline acetyltransferase (ChAT, EC.2.3.1.6) to synaptosomal membrane fragments is suggested to reflect distribution *in vivo* mediating the efficient acetylation of choline entering nerve terminals by sodium-dependent high affinity uptake (Atterwill & Prince, 1977, 1978a). Less efficient acetylations in chick brain (Atterwill & Prince, 1978b) and in squid optic lobe (Barker, Dowdall & Mittag, 1975) support this suggestion since ChAT in these species (pI, 5.2 to 6.4) lacks net positive charge (Atterwill & Prince, 1978b; Polsky & Shuster, 1976). Rabbit brain ChAT was known to bind strongly to membrane fragments (Tuček, 1966) and to cation exchange resins, although it also lacked net positive charge (pI, 6.9, Malthé-Sørensen, 1976). Investigation of the metabolism of [^3H]-choline in rabbit brain therefore seemed likely to further clarify the relationship between the molecular properties of ChAT, the affinity of the enzyme for

membrane fragments and its disposition *in vivo*.

Metabolism of [^3H]-choline in small slices of cortex was investigated as described previously (Atterwill & Prince, 1977; 1978a, b) using samples from male, New Zealand white rabbits (2–2.5 kg). Sodium-dependent uptake (SDU) of choline, calculated by correcting total uptake for that in sodium-free medium (SFU) accounted for 69% total uptake; 91% [^3H]-choline accumulated by SDU yielded ACh (Table 1). Acetylation of choline in nerve terminals therefore is apparently as efficient in rabbits as in rats, even though the net charge characteristics of ChAT in these species differ appreciably.

Net charge, therefore, may be an insufficient index of affinity for membrane if, for example, ChAT is asymmetric in charge distribution. The hydrophobic properties recently reported for rat (Rossier, 1977) and bovine brain ChAT (Malthé-Sørensen, Lea, Fonnum & Eskeland, 1978), however, may be representative of the enzyme from a variety of species, providing an alternative basis for interaction with membrane.

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Table 1 Metabolism of [^3H]-choline in rabbit cortical slices

	[^3H]-choline uptake		[^3H]-label recovered as		[^3H]-phosphoryl-choline ^a
	pmol g ⁻¹ 10 min ⁻¹	% total ^b	[^3H]-choline ^a	[^3H]-ACh ^a	
Total	191.1 \pm 7.6 ^c	100	36.2 \pm 2.4	61.5 \pm 2.2	2.3 \pm 0.1
SFU	59.0 \pm 3.1 ^c	30.9	85.0 \pm 0.9	8.0 \pm 0.8	7.0 \pm 0.4
SDU	132.1 \pm 7.0 ^d	69.1	8.6 \pm 3.4	90.9 \pm 3.4	0.4 \pm 0.1

^a[^3H]-metabolite expressed as the mean \pm s.e. mean of the average percentages it represented of the total recovered [^3H]-label, 4 animals, determinations in triplicate; one animal, determinations in duplicate; 30 mg tissue, 10 min incubation, 37°C.

^bPercentage of the mean total [^3H]-choline uptake.

^cThe mean \pm s.e. mean of 14 observations.

^dThe s.e. mean was calculated using the equation: $\text{var}(x-y) = \text{var}(x) + \text{var}(y) - 2.\text{covar}(x, y)$ (Colquhoun, 1971).

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The 'Electronic platelet aggregometer'

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We have invented a device which can be used to study platelet behaviour in whole blood as well as platelet rich plasma. An evaluation of this 'electronic aggregometer' will be the subject of a communication presented by us at this meeting. In this demonstration we will deal with the principle of its operation and method of use.

Citrated (or heparinized) blood or plasma samples (1 ml) are pipetted into siliconized glass cuvettes (of the type used in standard aggregometers) and placed into heated holders (37°C) and stirred at 600 rpm with a 'flea' magnet. A perspex cap that fits on top of the cuvette holder holds two electrodes – 0.25 mm diameter platinum wires 1.5 cm long, separated by approximately 1 mm. The electrode assembly projects into the sample to a depth of 1 cm and is energised by an oscillator generating a 15 kHz sine wave with an amplitude of 100 mv, which is passed through the blood between the electrodes. Electron microscopy showed that during the initial contact with the sample, the electrode becomes coated with a platelet

monolayer. In the presence of aggregating agents, however, platelets stick to the monolayer and progressively cover the electrode. Resultant changes in conductance cause a change in the excitation voltage across the cell and this is amplified, rectified and filtered before being fed via an outlet socket to any suitable chart recorder. The trace thus obtained is in many respects identical to that obtained with an optical aggregometer.

After aggregation has occurred the electrode assembly is removed and cleaned with a piece of tissue. The cuvette is rinsed with saline and the apparatus is then ready for another sample.

The operation of the machine depends upon efficient control of temperature and stirring rate, but it is extremely simple to use and is suitable for measuring the aggregation of platelets within 1–2 min of obtaining the blood sample. It is, therefore, well adapted for assay of labile endogenous hormones such as prostacyclin. The device is novel, although a similar principle has been used to measure clot formation (Amiram, 1970) in blood.

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Influence of mode of blood sampling on the immunoreactive insulin concentration in serum of *Bordetella pertussis*-treated mice

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Pertussis vaccine induces hyperinsulinaemia in mice and rats (Gulbenkian, Schobert, Nixon & Tabachnick, 1968) and augments the hyperinsulinaemia induced by various stimuli (Sumi & Ui, 1975). Hyperinsulinaemia was observed also in *B. pertussis* infection in mice (Pittman, Furman & Wardlaw, unpublished). A