importance in receptor complexing of the pentyltrimethylammonium ion.

The muscarinic potencies of pentyltrimethylammonium bromide, (±)-1-methyltrimethylammonium iodide and (±)-2-methyltrimethylammonium iodide were determined in vitro on the guinea-pig ileum, using Tyrode solution containing $1 \times 10^{-4} M$ hexamethonium dichloride to dispel ganglion stimulant contributions to the measured responses. Drug responses were completely abolished when the compounds were administered in the presence of $1 \times 10^{-7} M$ atropine sulphate[7].

Nicotine-like stimulant actions of the compounds were conducted on the rectus abdominis muscle of the frog *Rana pipiens*[8]. Carbachol was used as the standard drug in all experiments and equipotent molar ratios were determined by complete four-point assays.

Although the pentyltrimethylammonium salts are less active than the standard, carbachol, the results clearly demonstrate that the nicotinic and muscarinic receptors can differentiate between the substituted aliphatic agonists (Table). The pentyltrimethylammonium and 1-methylpentylammonium salts are virtually equipotent nicotinic stimulants whereas 2-methylpentyltrimethylammonium iodide is considerably less active on a molar basis. The reverse situation holds true at the muscarinic receptor where 2-methylpentyltrimethylammonium iodide and pentyltrimethylammonium bromide are more potent than the 1-methylpentyltrimethylammonium salt.

The consistancy between the above results and those previously determined for the analogous acetylcholines[5,9] implies that the pentyltrimethylammonium ion fits the cholinergic receptors in a similar way to acetylcholine. Moreover, the quantitative variations noted (Table) indicates that the muscarinic receptor is more sensitive to exchange of the ester group for methylenes than the nicotinic receptor[10].

Equipotent molar ratios relative to carbachol

	Frog rectus abdominis (Nicotinic)	Guinea-pig ileum (Muscarinic)
Carbachol	1.00	1.00
Pentyltrimethylammonium bromide	2.14 ± 0.080	15.94 ± 2.58
(±)-1-Methylpentyltrimethyl ammonium iodide	2.89 ± 0.423	300.2 ± 20.60
(±)-2-Methylpentyltrimethyl ammonium iodide	140.1 ± 16.20	10.68 ± 0.372

Each experiment was repeated 3 times. The results show the equipotent molar ratios and the standard errors of the means.

Résumé. Étude des propriétés cholinergiques des iodures du méthyl-1 et du méthyl-2-pentyltriméthylammonium. Dans leur comportement agonistique, ces sels offrent des variations parallèles à celles de dérivés méthyles de l'acétylcholine. Le cation pentyltriméthylammonium est donc capable de s'associer aux récepteurs cholinergiques tels que l'acétylcholine.

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Removal of Acetylcholine During Perfusion of Liquor-Spaces and its Influence on Outflow Volume

Acetylcholine (Ach) appears in the effluent during perfusion of the cerebroventricular system[1] and intermeningeal spaces of the spinal cord with an anticholinesterase[2]. There is, however, not much information concerning the fate of Ach in these spaces. Bhawe[3] recovered 56% from cat cisterna magna after a single intraventricular injection of Ach. In the present work[4], Ach was perfused continuously through different compartments of CSF-containing spaces and its disappearance studied.

Methods. 26 cats of either sex were anaesthetized by an i.v. injection of 25 mg/kg sodium pentobarbital. The liquor-containing spaces were perfused as follows: a) the cerebral subarachnoid space, from the parietal cortex to cisterna magna[5]; b) the spinal subarachnoid space, from the cisterna magna to lumbosacral foramen[2]; c) the cerebroventricular system[1], from the left lateral ventricle either to cisterna magna or d) to aqueduct of Sylvius. Acetylcholine chloride (100 ng/ml) was added to the perfusion fluid[6] which was introduced at a rate of 0.1 ml/min. The content of Ach was checked by biological assay at the beginning and end of the experiment to exclude loss due to spontaneous hydrolysis. 20 min samples of perfusate were collected in graduated tubes and Ach content determined on guinea-pig isolated ileum[7]. Disappearance of Ach was calculated by comparing inflow-outflow amounts. In a separate series of experiments, pieces of dura mater and arachnoid were dissected from different regions of brain and spinal cord and cholinesterase activity was determined histochemically[8], as well as colorimetrically[9] using homogenates.

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In 3 experiments, a branch of a choroidal artery of 4th ventricle-plexus choroides was observed through a dissection microscope and its diameter measured before and after local application of a drop of the perfusion fluid containing Ach. Due to difficulties in focussing, it was not possible to assess simultaneously the diameter of more than one vessel.

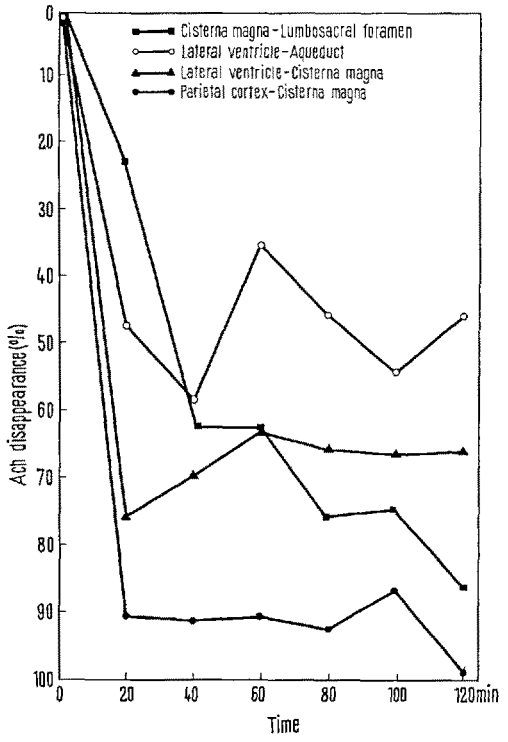

Fig. 1. Disappearance of Ach during perfusion of liquor-spaces with fluid containing 100 ng/ml of Ach. Values represent means of 5 experiments in each group.

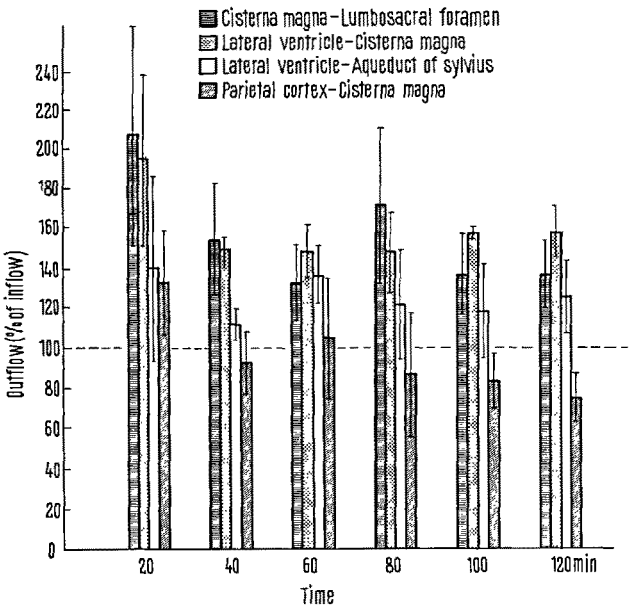

Fig. 2. Inflow/outflow ratios during perfusion of liquor-spaces with fluid containing 100 ng/ml of Ach. Values represent means of 5 experiments in each group. Vertical bars indicate S.D. and broken line, inflow volume.

Results. Figure 1 shows the mean results concerning the disappearance of Ach during 2 h perfusion. Ach was most efficiently removed from the cerebral subarachnoid space and least from the ventricular system. This latter finding confirms previous work[1]. The rate of disappearance of Ach from the spinal subarachnoid space was small at the beginning and increased gradually throughout the experiment.

No demonstrable cholinesterase activity was observed in meninges up to 6 h of incubation with acetylthiocholine. In contrast, after 30 min of incubation, dark spots, corresponding with motor endplate region of mice diaphragm used as control, were seen. Moreover, no Ach was split when incubating 100–300 mg of meninges homogenate with 4 m*M* of Ach during 2 h at 25 °C.

Throughout the perfusions, there were differences between inflow and outflow volumes. In Figure 2 are shown the inflow/outflow ratios of various samples collected during 2 h perfusion of different segments. Calculating the total volume of 6 samples, the effluent increased by 55% in c), 40% in b) and 15% in d). On the other hand, in a) total outflow volume equalled that of inflow, although the last samples collected were small. This would indicate some absorption of perfusion fluid.

A few seconds after local application of Ach, diameter of choroidal artery branch increased considerably (Table). The vessel remained dilated for at least 3 min after washing the plexus with perfusion fluid without Ach.

Discussion. It seems reasonable to postulate that Ach removal from liquor-containing spaces could be due to 1. passage into blood stream, 2. uptake by nervous tissue surrounding the spaces and 3. destruction by cholinesterase. The relative importance of each one of these factors could differ according to the compartment considered. Mechanism 2 and 3 could account for the greatest disappearance from cerebral subarachnoid space, as the cerebral and cerebellar cortices are known to be rich in cholinesterase[10] and the cerebral cortex is able to incorporate Ach[11]. In contrast, enzymatic destruction could have played a minor role in the cerebroventricular system, as previous work[12] showed that lining ependyma contain no detectable cholinesterase. When the outflow was from the cisterna, a part of the cerebral subarachnoid space was also included in the perfusion and this could explain the better removal of Ach.

It may be interesting to note that the pattern of disappearance of Ach from the spinal subarachnoid space was conspicuously different from other regions. The

Diameter of a choroidal artery branch of 4th ventricle-plexus choroides a) before and b) after topical application of Ach

Animal No.	Diameter (μm)	
	a)	b)
1	30	50
2	20	40
3	25	45

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spinal white matter possesses histochemically demonstrable cholinesterase activity[13], although presumably low and therefore only small amounts of Ach could have been destroyed during perfusion. Moreover, the present experiments failed to demonstrate cholinesterase activity in cat meninges, and in humans it appeared tenuously and only in nerve fibers accompanying vessels[14]. In view of the gradual clearance of Ach, one could speculate that it could have been removed by an active transport mechanism similar to that reported for iodide[15].

The increase of outflow volume could be attributed to vasodilatation and/or increased permeability elicited by Ach on plexus choroid vessels. This view is supported by the higher outflow obtained from c), which included all the plexuses choroides, and by the lower outflow from d) where the 4th ventricle was excluded. It may also be possible that during b), some Ach reached the 4th ventricle, and this could explain the increase in outflow. It should be recalled that, in dog, the plexus choroides of 4th ventricle produce two thirds of the total CSF[16]. The possibility that Ach acts on non-choroidal CSF-secreting structures, such as ventricular ependyma[1,17] and thus contributing to the increase of effluent, cannot be ruled out.

On the other hand, the reduction of outflow from a) could have been due to the fact that no plexus choroides came in contact with Ach. An additional reason could be the action of choline, which must have been produced as a result of Ach destruction and is known to reduce[18] CSF production[19].

Zusammenfassung. Bei Perfusion der Liquorräume im Katzengehirn verschwindet Acetylcholin zu 90% aus dem Subarachnoidalraum, zu 60% aus den Ventrikeln. Eine fortlaufende Abnahme wurde im spinalen Subarachnoidalraum beobachtet. Acetylcholin erweiterte den Ast der Choroidarterie und vergrösserte stark das Abflussvolumen aus Ventrikeln und dem spinalen Subarachnoidalraum.

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Beeinflussung mikrosomaler Enzyme durch Tetrachlorkohlenstoff mit und ohne Phenobarbitalvorbehandlung[1]

Tetrachlorkohlenstoff führt bei Versuchstieren und bei exponierten Personen zu erheblichen Leberschäden. Der genaue Mechanismus der akuten Schädigung ist bisher noch nicht bekannt. Verschiedene Befunde sprechen dafür (McLean[2], Sasame[3] und Smuckler[4]), dass der Tetrachlorkohlenstoff zu einem aktiven Metaboliten abgebaut wird.

Wir untersuchten, ob die toxische Wirkung des CCl_4 in einem Zusammenhang mit den mikrosomalen Arzneimittel-metabolisierenden Enzymen und anderen mikrosomalen Enzyme steht, und ob sich die Toxizität nach Phenobarbitalvorbehandlung verstärken lässt.

Unsere Untersuchungen wurden 3 h nach oraler Gabe des CCl_4 durchgeführt, nachdem Dingell und Heimberg[5] zeigen konnten, dass 2–3 h nach oraler Gabe von $^{14}CCl_4$ in der Mikrosomenfraktion die höchste Aktivität nachweisbar ist.

Männliche 140–160 g schwere Wistar-Ratten wurden mit Altromin-Standardfutter und Wasser ad libitum ernährt. Eine andere Gruppe erhielt ausserdem 3 Tage lang je 80 mg/kg Körpergewicht Phenobarbital-Natrium i.p. injiziert. Am 4. Tag bekamen die Tiere nach 12stündigem Nahrungsentzug verschiedene Mengen CCl_4 (1:1 in Olivenöl gelöst) durch eine Schlundsonde verabreicht. Die Kontrolltiere beider Gruppen bekamen entsprechende Mengen Olivenöl.

Die Mikrosomenpräparation führten wir nach Remmer[6] durch und untersuchten folgende Enzyme: Das Cytochrom b_5 und das Cytochrom P_{450} nach Omura und Sato[7], die Desmethylase und die Hydroxylase, wobei wir das in vitro gebildete Formaldehyd bzw. *para*-Aminophenol nach Schenkmann[8] bestimmten, die NADPH-Cytochrom-c-Reduktase nach Phillips et al.[9] und die Glukose-6-Phosphatase nach Harper[10]. Ausserdem bestimmten wir die Bindungsfähigkeit des endoplasmatischen Reticulums für Anilin und Hexobarbital nach Schenkmann et al.[8].

Einige der von uns untersuchten Enzyme, so das Cytochrom P_{450}, die Desmethylase, die Hydroxylase und die Glukose-6-Phosphatase, zeigten nach CCl_4-Gabe eine dosisabhängige, statistisch signifikante Aktivitätsabnahme (Tabellen I und III). Das Cytochrom b_5 und die

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