Syrian hamster cells by human adenovirus type 12.

A significant question yet to be resolved is why *in vitro* transformation by adenovirus type 12 has not been observed frequently before this time. Certainly many laboratories have been striving to obtain this result. Kitamura *et al*(3) mention in passing an apparent *in vitro* transformation, but such results have yet to be published. It would appear that the procedures chosen for this investigation circumvented barriers imposed by the techniques employed by others, which prevented the necessary virus-cell association required for transformation. Investigations are now in progress to determine which variables in the test system are significant for consistently obtaining *in vitro* transformation by this virus.

Summary. *In vitro* transformation by human adenovirus type 12 of cells derived from newborn hamster kidneys is described. Such transformation was obtained in 3 different types of kidney cell cultures: trypsinized cells, tissue fragments in plasma clots, and tissue fragments separated from the culture medium by a continuous cellophane membrane. This transformation included morphological alteration of the cells, recovery of the ability to divide, and development of an adenovirus type 12 specific complement-fixing antigen without the presence of detectable infectious virus. The transformed cells did not react with SV-40 specific fluorescein tagged antibodies. The malignant potential of these cells *in vivo* is under test. It is anticipated that the procedures employed in these experiments will offer new prospects for the study of human viral oncogenesis.

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Histamine Metabolism in the Brain of Conscious Cats.* (29061)

B. JONSON AND T. WHITE (Introduced by G. Kahlson)
Institute of Physiology, University of Lund, Sweden

Histamine is present in the brain(1,2), and the metabolism of histamine in the brain of anaesthetized cats has previously been studied by perfusion of the cerebral ventricles with radioactive histidine and histamine(3, 4). In the present experiments these radioactive substances were injected into the cerebral ventricles of conscious cats, thereby avoiding possible effects of anaesthesia and of the dissection of the brain for ventricular perfusion.

Methods and materials. A Collison cannula was implanted into the lateral ventricle of 5 cats, as described by Feldberg and Sherwood(5). The injections through the cannula of radioactive substances were made 9 days, or longer, after the implantation. The substances were dissolved in 0.2 ml Tyrode solu-

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TABLE I. Catabolism of C^{14}-histamine in Cat Brain After Injection into the Lateral Ventricle.

Exp	Time after injection (hr)	C^{14}-histamine injected (μg)		% of injected C^{14}-histamine recovered as			
				Histamine	Methyl-histamine	Methylimidazole-acetic acid	Imidazole-acetic acid
1	1	10	In brain	.5	16	13	.5
			In 1 ml CSF	.2	2.9	.4	<.2
2	2	10	In brain	.6	16	22	.8
			In 1 ml CSF	.2	1.4	.3	<.2
3	1	2	In brain	3.0	23	17	—*
			In 1 ml CSF	< .2	1.1	.2	<.2

* Sample lost.

tion and washed in by another 0.2 ml Tyrode solution. The injections had little effect on the behavior of the animals: only a few licking or swallowing movements were observed similar to those observed after Tyrode solution alone(6). One or two hours after injection the cat was bled to death under ether anaesthesia. Cerebrospinal fluid was collected from the cisterna magna. Bromphenol Blue solution was injected through the intraventricular cannula. The correct position of the cannula was checked by dissection, and by inspection of the distribution of the dye in the ventricular system. The walls of the cerebral ventricles were blotted with filter paper, and the brain was homogenized in 0.1 N HCl. Samples of the homogenate (usually one-fifth of the total) and of the cerebrospinal fluid were taken for analysis of their content of radioactive histamine and its metabolites, using the isotope dilution methods devised by Schayer (for details of the chemical procedures and measurements of radioactivity, see 3,4). The radioactive drugs used were C^{14}-histamine and C^{14}-histidine, both labelled in the 2-position of the imidazole ring. Under the conditions of the assay, 1 μg of the C^{14}-histimine base gave 3000 counts/min. The C^{14}-histidine solution used for injection was analyzed for its content of C^{14}-histamine, and it was calculated that in Exp. 1, Table II, an amount of C^{14}-histamine equivalent to 2100 counts/min was injected. In Exp. 2, Table II, where a purified sample of C^{14}-histidine was used, the corresponding figure was 115 counts/min. Apart from this, the solutions injected did not contain significant amounts of the radioactive substances that were measured in the different experiments.

Results. The results are given in Tables I and II. Table I shows that very little unchanged histamine remained in the brain one and two hours after injection. Instead, the brain contained methylhistamine and methylimidazoleacetic acid, formed from histamine by ring-N-methylation. Only minute amounts of imidazoleacetic acid were found. The cerebrospinal fluid contained little methylimidazoleacetic acid, but considerably more methylhistamine.

Table II shows that both histamine and methylhistamine were formed from injected histidine. In these experiments approximately equal amounts of the two substances were found in the brain, in contrast to the findings after an injection of histamine (Table I). Neither of the two substances could be demonstrated in the cerebrospinal fluid after histidine injection.

At the end of one experiment (Exp. 3, Table I) bladder urine was analyzed for radioactive methylimidazoleacetic acid, the main urinary metabolite of histamine in the cat(6,7). The total amount in urine corresponded to 2.5% of the histamine administered, showing that in these experiments very

TABLE II. C^{14}-histamine and C^{14}-methylhistamine in Cat Brain 2 Hours After Injection of C^{14}-histidine into the Lateral Ventricle.

Exp	C^{14}-histidine injected (μg)	C^{14}-histamine (counts/min)	C^{14}-methyl-histamine (counts/min)
1	630	270	330
2	390	250	250

little of the histamine injected into the cerebral ventricles, and its metabolites, was absorbed into the blood stream. This is in agreement with earlier findings obtained with somewhat different techniques(8,9).

Discussion. The present results support the view that histamine administered to the brain is rapidly methylated by the enzyme histamine-N-methyl transferase(10), and then, in part, further oxidized to methylimidazoleacetic acid, whereas histamine formed in the brain from histidine is less rapidly methylated(3). Such a difference in the extent of methylation between "exogenous" and endogenous" histamine was not observed by Bjurö, Westling and Wetterqvist(11) in the urine of intact rats after subcutaneous injections of radioactive histamine and histidine, respectively. This suggests that, disregarding possible species differences, the metabolism of histamine in the brain is quantitatively different from the metabolism of histamine in the rest of the body. This may be related to the findings that histamine penetrates poorly, or not at all, from the blood into the brain(12), and that histamine administered to the brain, and its metabolites there, are absorbed into the blood stream only to a very small extent, as mentioned above. The present results in conscious cats agree fairly well with observations on the metabolism of histamine and histidine administered to the brain by ventricular perfusion in anaesthetized cats (3,4).

Summary. C^{14}-histamine and C^{14}-histidine were injected into the cerebral ventricles of conscious cats. After one to two hours C^{14}-histamine and its metabolites in the brain were determined. The formation of histamine from histidine, and methylhistamine from histamine, were demonstrated. The results indicate that in the brain "exogenous" histamine is more rapidly methylated than "endogenous" histamine.

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Evidence for Cholinergic Transmission at a Central Synapse.* (29062)

D. T. FRAZIER AND L. L. BOYARSKY (Introduced by L. D. Carlson)
Department of Physiology and Biophysics, University of Kentucky Medical Center, Lexington

A large amount of evidence exists involving acetylcholine as a synaptic transmitter in the central nervous system(1). The experiments reported here employ the hemicholinium compound HC-3 to test the cholinergic properties of synapses in the cuneate nucleus, the first relay nucleus in the afferent pathway for the sensation of touch. HC-3, first synthesized and investigated by Schueler(2), has been shown to interfere with the synthesis of acetylcholine in sympathetic ganglia and minced brain(3). It has also been demonstrated that choline can antagonize the effects of HC-3(2). Several workers have

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