Table 1. Ratio of optical densities of dimer bands to those of monomer bands. Observed and calculated ratios are listed for various mixtures of 2′,3′-benzylidine-5′-trityl derivatives of guanosine (G) and cytidine (C), 9-ethyl adenine (A) and 1-cyclohexyl uracil (U) in chloroform at 25°C. The upper figure in each square is the observed ratio of the optical density of the dimer band to that of the monomer band. The dimer band used is that which appears between the symmetric and antisymmetric stretching bands of the monomer. The lower figure (in parenthesis) is the calculated ratio which is obtained from the sum of the optical densities of the individual (unmixed) solutions at half the total concentration. The observed and calculated ratios are similar in all cases with the exception of those determined for the interaction between the guanosine and cytidine derivatives and between the adenine and uracil derivatives. The results are presented for two different total concentrations.

Derivative	Ratio of optical densities for derivatives		
	C	U	G
*Total concentration, 0.0016*M			
A	0.20 (.21)	0.32 (.23)	0.48 (.50)
G	3.7 (.56)	1.2 (1.1)	
U	0.27 (.28)		
*Total concentration, 0.0080*M			
A	0.30 (.29)	0.55 (.23)	0.53 (.55)
G	5.4 (.70)	1.3 (1.4)	
U	0.34 (.32)		

tually identical to the sum of the two individual spectra. These two molecules are present in solution, but they do not associate with each other to any observable extent. This is remarkable since both adenine and guanine have donor protons and electronegative atoms which are capable of forming hydrogen bonds. However, there is no preferential association.

Table I, listing the ratio of the optical density of an association band to that of a monomer band, gives the results of all possible mixtures. For example, in the spectrum of the mixed guanosine and cytidine derivatives (Fig. 5), this ratio is that of the optical density of the mixed dimer band at 3488 cm^{-1} to the optical density of the monomer band at 3533 cm^{-1}. The ratio of the analogous optical densities obtained by adding the optical densities of the separate solutions at half concentration is also given. The two ratios would be equal in all cases if there were no interaction other than self-association. These calculated and observed ratios are substantially the same for mixtures of derivatives of adenine with cytosine, adenine with guanine, adenine with cytidine, guanine with uracil, and cytosine with uracil. However, a substantial difference between observed and calculated ratios is seen in the mixtures of the derivatives of adenine and uracil and of those of guanine with cytosine.

These results together with those of NMR studies clearly illustrate the fact that adenine and uracil (or thymine) derivatives associate with each other selectively, as do derivatives of guanine and cytosine, a feature of the selectivity found in the nucleic acids themselves. One cannot directly relate the studies in nonaqueous solution to the interactions found in aqueous solutions, but since the interior of the double-stranded DNA molecule is largely hydrophobic, it is not unreasonable to assume that this type of interaction operates within the DNA molecule.

It is widely believed that complementary pairing of purines and pyrimidines in the nucleic acids is largely due to the geometry of the polynucleotide backbone, since these pairs alone give rise to a unit which is able to fit into a regular double-stranded helix. However, now we would say that the double-helical form of the nucleic acid molecule represents a combination of both geometrical requirements and other stabilizing features which we have observed. It has been suggested (*2*) that forces between dipoles or between dipoles and induced dipoles arising within the unsaturated purine and pyrimidine rings may contribute significantly to the specificity of these interactions. Further investigation will be necessary before the nature of these forces is completely elucidated.

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9. Recently we have learned of an infrared study [J. Pitha, R. N. Jones, P. Pithova, *Can J. Chem.* **44**, 1045 (1966)] of other derivatives of guanosine and cytosine in chloroform solution. Their results are consistent with the observations reported here and with those of NMR studies.
10. This research was supported by grants from the NSF, ONR, USAF and NASA.

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Anticholinergic Blockade of Centrally Induced Thirst

Abstract. *A cholinergic antagonist applied to many of the limbic and diencephalic structures, cholinergic stimulation of which induces thirst, prevents drinking in response to cholinergic stimulation of other parts of the system. Thus, the elicitation of drinking after chemical stimulation of a particular site seems to be dependent on the functional state of other structures in the circuit.*

The application of a cholinergic drug to many parts of the brain induces thirst and ingestion of water in the rat, while an adrenergic drug applied to many of the same loci induces hunger and ingestion of food (*1*). Hernández Peón and his associates, working on the cat, have traced a hypnogenic or "sleep circuit" that is triggered by cholinergic drugs and that can be blocked by application of atropine (a cholinergic antagonist) to parts of the system caudal, but not rostral, to the site of cholinergic stimulation (*2*). Neural systems mediating many of these biological drives appear to follow generally parallel paths through limbic and diencephalic structures. Thus, neural specificity (and separation) of function may be determined by a chemical coding system as well as by contiguity of synaptic connections.

Fisher (*3*, *4*) has postulated that the neural basis for the thirst drive consists of complex alternative and reciprocal pathways of neurons susceptible to activation by cholinergic stimulation. Such a system could increase the duration of the drive by supporting reverberatory firing patterns. Also, alternative neural pathways would be available to insure continued function after injury to certain components of the system. The hypothesis provides one explanation for the fact that lesions in many of the brain structures,

cholinergic stimulation of which induces drinking, do not disrupt the normal pattern of thirst.

If the relationships between the structures within the cholinergic thirst system are essentially quantitative and alternative pathways are available, then blocking one component with a cholinergic antagonist should not prevent the induction of drinking by simultaneous cholinergic stimulation of another part of the system. On the other hand, if structures in the system subserve unique functions related to the thirst drive, then a functional lesion (cholinergic blockade) of such structures should prevent the cholinergically induced drinking response.

The hypodermic guide shafts of permanent brain implants were stereotaxically aimed for two separate brain sites of each male hooded rat. The animals were then kept in their home cages where food and water were always available. After recovering from surgery, animals were tested at 4-day intervals in special cages containing a Plexiglass drinking well attached to a 50-ml burette. Water intake was measured during the hour before testing and then again during the hour after insertion of 1 to 3 μg of crystalline chemical into target areas below one or both implant shafts (*3*).

Choline chlorine carbamate (Carbachol) and atropine were the cholinergic and anticholinergic agents injected into all animals in the study. Other chemicals, including sodium chloride, phenoxybenzamine (an adrenergic antagonist) and scopolamine (a cholinergic antagonist) were tested in selected animals.

Initial tests of each animal determined whether drinking occurred after points below each of the two implanted guide shafts were stimulated with carbachol (drinking sites). If two drinking sites were located, then we simultaneously injected carbachol into one structure and atropine into the other. Whenever drinking was blocked, carbachol alone was used on a subsequent test day to assure that drinking was still triggered. If the animal was not induced to drink, the evidence for inhibition of drinking by atropine was considered invalid. If drinking was elicited, however, the sites of atropine and carbachol injection were reversed, and the above test sequence was repeated.

Table 1 presents data gathered from 28 animals in which stimulation of both neural sites resulted in drinking. Introduction of atropine into a number of drinking loci prevents drinking in response to cholinergic stimulation of other structures studied, and the blockade is bidirectional. The blockades which occurred between septal nucleus and contralateral anterior thalamus and between left and right septal nuclei are of particular interest since the demonstration of a blocking effect in these cases suggests that drinking in response to stimulation with a cholinergic drug is dependent on the functional state of structures within the contralateral hemisphere.

Table 1. Blockade by atropine of cholinergically induced drinking.

Structure receiving carbacol*	Structure receiving atropine*	Mean water intake (ml)
SN	None	11.7
CG	None	11.3
FX	None	13.4
MT	None	9.6
AT	None	13.3
MM	None	11.3
SN	FX	1.2
SN	MT	2.3
SN	AT	3.5
SN	AT (contralateral)	2.3
SN	MM	1.0
CG	MM	0.1
FX	SN	1.2
FX	MM	0.4
MT	SN	0.5
AT	SN	2.8
AT	SN (contralateral)	2.4
MM	SN	4.0
MM	CG	0.1
MM	FX	3.4

* SN, septal nucleus; CG, cingulate gyrus; FX, fornix; MT, medial thalamus; AT, anterior thalamus; MM, medial midbrain. The group of 28 animals consisted of two with implants in SN and FX, five with implants in SN and MT, three in SN and AT, five in SN and contralateral AT, four in SN and MM, five in contralateral SN, two in CG and MM, and two in FX and MM.

A number of control tests have been performed on these and other animals. We find that sodium chloride applied to a drinking site does not mimic the blockade produced by atropine of drinking in response to cholinergic stimulation of another structure; that phenoxybenzamine blocks on occasion, but not reliably; that scopolamine blocks as well as atropine; and that atropine introduced into structures negative for carbachol-induced drinking does not block drinking induced by cholinergic stimulation of positive drinking structures.

The daily water intake of all animals in which measures were taken was normal after a blockade with atropine had been demonstrated.

These data indicate that under the stated conditions all of the tested components of the postulated thirst drive system must be functional if drinking is to be elicited by cholinergic stimulation elsewhere in the system.

There are several reasons why caution should be used in attributing the blockade specifically to anticholinergic action. First, phenoxybenzamine occasionally produced a partial or complete block, which may or may not be due to a nonspecific disruptive action. Nonspecific depressant or disruptive effects of atropine cannot be completely ruled out either, although Fisher (*3*) and Grossman (*5*) have both produced evidence suggesting that adrenergic triggering of eating behavior is actually enhanced by atropine, while drinking is suppressed. Even if the blockade were nonspecific in nature, however, the data still suggest that disturbances of the functional integrity of certain structures in the "thirst circuit" interfere with the initiation of cholinergically induced drinking.

Our results imply that structures in the thirst system subsume different functions, or at the very least, that they all must be operational when drinking is cholinergically induced. However, many reports indicate that extensive lesions in most of these structures do not interfere with thirst induced by deprivation of water. Two possibilities suggest themselves: (i) disruption of circuits due to lesions or blockade may be quite temporary; and (ii) the major differences in the neural events occurring during drinking induced by water deprivation and that induced cholinergically may involve the input from peripheral feedback and central triggering mechanisms (that is, receptors in the throat, osmo- and temperature receptor neurons, and so forth) that function only during deprivation. Sustained input from such sources (during deprivation) may permit the system to function even while one or more component structures are out of order. In the absence of such input, however, cholinergic stimulation alone may not be sufficient to sustain activity in an incomplete (atropine-blocked) circuit.

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Reovirus-Specific Polyribosomes in Infected L-Cells

Abstract. *Polyribosomes were isolated from reovirus-infected L-cells. Virus-induced RNA isolated from these polyribosomes was single-stranded, heterogeneous in size, and capable of extensive hybridization with viral double-stranded RNA. Reovirus RNA, therefore, specifies in infected cells the formation of a single-stranded messenger RNA for virus-specific protein synthesis.*

Reovirus is unique among mammalian viruses and bacteriophages in that its genetic material is double-stranded RNA (*1*). Very few features about its replication have been established. Some 7 hours after infection of L-cells, a virus-induced synthesis of RNA commences, and this synthesis probably involves prior formation of at least two new proteins (*2*). Approximately 40 percent of the newly synthesized RNA is double-stranded and resembles the RNA of the virion in physical characteristics; the remaining RNA is single-stranded and heterogeneous in size, being distributed between 30*S* and 10*S* molecules in sucrose-gradient analyses (*3*). We now describe experiments showing that part of this single-stranded RNA is associated with polyribosomes in infected cells and is probably a messenger RNA (mRNA) involved in virus-directed protein synthesis.

Before infection, exponentially growing suspension cultures of L-cells were centrifuged; the cells were suspended to a concentration of 5×10^5 per milliliter in Eagle's minimal medium containing 2 percent bovine fetal serum. After the culture was incubated for 1 hour, actinomycin D (0.5 μg/ml) was added, and the culture was incubated 1 hour longer to ensure suppression of host RNA synthesis. A stock suspension of reovirus, type 3, was then added to give a multiplicity of infection of 5 to 10 plaque-forming units (PFU) per cell (*3*).

For isolation of polyribosomes from infected or uninfected cultures, approximately 10^8 cells were centrifuged, washed three times with ice-cold phosphate-buffered saline (*4*), and resuspended in 1.5 ml of extraction buffer containing 0.01*M* tris-HCl at *p*H 7.4, 0.1*M* NaCl, and 0.015*M* $MgCl_2$. All procedures were carried out at 4°C. After 15 minutes the detergent, BRIJ-58, was added to a final concentration of 0.5 percent. The cells were then ruptured in a tight-fitting Dounce homogenizer, and nuclei were removed by centrifugation at top speed in a clinical centrifuge. Sodium deoxycholate was added to the supernatant cytoplasmic fraction to a final concentration of 0.5 percent. The material was then layered on a 15- to 30-percent linear sucrose gradient prepared in the same buffer used for extraction, and centrifuged for 110 minutes at 24,000 rev/min and 4°C in a Spinco centrifuge. Under these conditions polyribosomes were present in fractions 5 to 22 (Fig. 1), while virions were sedimented to the bottom of the tube.

Functional polyribosomes in reovirus-infected cultures were demonstrated by treating the cultures with C^{14}-labeled algal-protein hydrolyzate for 5 minutes, at 10.5 hours after infection. The cytoplasmic fraction was then prepared and analyzed by sucrose-gradient sedimentation (Fig. 1).

Few polyribosomes were seen by optical-density measurements in uninfected cultures after treatment with actinomycin D, and there was only a small increase in the number after infection. Moreover, in the uninfected culture only a small amount of label was observed in the polyribosome region.

In contrast, a significant amount of label was seen in the polyribosomes of infected cells, an indication that viral-directed protein synthesis was in-

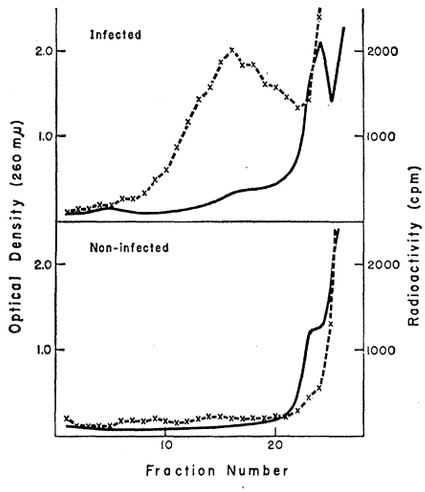

Fig. 1. C^{14}-Amino acid incorporation into polyribosomes. At 9.5 hours after infection, 200 ml of cell culture (5×10^5 cell/ml) were centrifuged, and the cells were resuspended in 50 ml of growth medium containing 1/40 of the normal amino acid concentration and 2 percent fetal calf serum. After incubation for 1 hour at 37°C, 50 μc of C^{14}-labeled algal-protein hydrolyzate was added. Uptake of label was stopped 5 minutes later by rapid chilling of the cells, and the cytoplasmic fraction was prepared and analyzed by sucrose-gradient sedimentation. Optical density in the gradient was monitored continuously with a Gilford recording spectrophotometer. Fractions collected from the gradient were precipitated with ice-cold trichloroacetic acid on glass-fiber filters, and the radioactivity of the filter was determined by liquid scintillation counting in a Tricarb spectrometer. Optical density (——); Radioactivity (x - x - x).

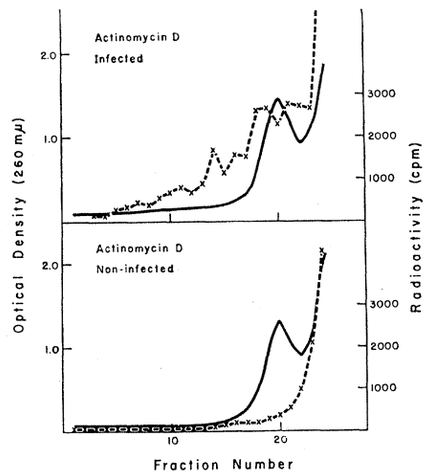

Fig. 2. H^3-Uridine incorporation into polyribosomes. Ten hours after infection, H^3-uridine (4770 μc/μmole, final concentration, 1 μc/ml) was added to 200 ml of cell culture. After 30 minutes, uptake of label was terminated by rapid chilling of the culture; the polyribosomes were then isolated. Optical density (——) and radioactivity (x - x - x) were determined as described in Fig. 1.