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Messenger Ribonucleic Acid of Cerebral Nuclei

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1. RNA was isolated from crude nuclear preparations and from ribosomes derived from rat brain and liver. Nuclear RNA was obtained by lysis of the nuclei with sodium dodecyl sulphate, followed by denaturation and removal of DNA and protein with hot phenol. 2. Base composition analyses indicated that the cerebral nuclear RNA preparation contained a higher proportion of non-ribosomal RNA than the analogous hepatic preparation. 3. Sucrose-density-gradient analyses revealed a heterogeneous profile for each nuclear RNA preparation, with two major peaks possessing the sedimentation properties of ribosomal RNA (18s and 28s). 4. Template activities of both preparations were widely distributed through the sucrose density gradients. 5. The cerebral nuclear RNA preparation was more active than the hepatic nuclear RNA preparation in promoting amino acid incorporation in cell-free systems from *Escherichia coli* and rat brain. 6. Cerebral nuclear RNA stimulated amino acid incorporation in a cerebral ribosomal system even in the presence of an excess of purified *E. coli* transfer RNA. 7. It is concluded that a significant proportion of cerebral nuclear RNA has the characteristics of messenger RNA.

Characterization of cerebral messenger RNA is of interest in view of the attention currently being focused on the possible role of protein synthesis in the specialized functions of the brain (see review by Roberts, 1966). Because of the heterogeneous nature of mammalian messenger RNA species in general and their sensitivity to degradation during preparation (Decken, 1965), little is known about concentrations, ranges of molecular sizes and turnover times of these moieties. The assumption has frequently been made that the rapidly labelled fractions of mammalian RNA after administration of radioactive RNA precursors represent messenger RNA species. Recent studies of this nature dealing with cerebral RNA have been reported (Jacob, Stevenin, Jund, Judes & Mandel, 1966; Egyhazi & Hydén, 1966*a*). However, the criterion of rapid labelling, which was originally derived from bacterial studies, is of doubtful applicability in mammalian systems where there is evidence for long-lived messenger RNA species (Seed & Goldberg, 1963; Revel & Hiatt, 1964; Greengard, Gordon, Smith & Acs, 1964). A further limitation of RNA-turnover experiments in mammalian tissues is provided by the observation that the RNA synthesized by isolated rat liver nuclei consists of 95% ribosomal RNA and only a small amount of DNA-like RNA (Blackburn & Klemperer, 1966). Mammalian messenger RNA species are probably best identified and assayed on the basis of their stimulatory activity on amino acid incorporation in isolated ribosomal systems.

The present report presents information on the characteristics of RNA extracted from crude preparations of rat brain nuclei by a procedure using hot phenol, which is based on the methods described by Georgiev & Mantieva (1962) and Scherrer & Darnell (1962). The data reveal that this cerebral nuclear RNA has a wide range of sedimentation values, a distinctive base composition, and considerable messenger activity in both bacterial and cerebral cell-free amino acid-incorporating systems.

METHODS

Preparation of nuclear and ribosomal RNA. Young adult male rats from an inbred Sprague–Dawley strain were used in these investigations. The animals were maintained on Purina laboratory chow *ad lib.* until they weighed 140–180g. They were then lightly anaesthetized with sodium pentobarbital (Nembutal) and exsanguinated from the abdominal aorta. Whole brains (and sometimes livers) were rapidly removed, placed in 12vol. of cold 0·32M-sucrose, and homogenized by hand with a glass homogenizer with a Teflon pestle. The homogenate was filtered through four layers of cheesecloth and the filtrate was centrifuged at 400*g* for 10min. Phase-contrast microscopy showed that the pellets obtained consisted largely of cerebral nuclei

with some contamination by myelin, erythrocytes and mitochondria. The crude nuclear preparation from about 30g. of tissue was suspended in 50ml. of 1% sodium dodecyl sulphate containing 3mM-EDTA and 0·01M-sodium acetate (pH 5·2); 25ml. of phenol (Mallinckrodt) saturated with this sodium dodecyl sulphate solution was added. The mixture was shaken in a water bath at 65°, first slowly for 4min. and then rapidly for 4min. Next, the suspension was cooled rapidly and centrifuged in the Sorvall centrifuge for 10min. at 8° and 35000*g*. The slightly viscous supernatant was pipetted off, 25ml. of phenol was added to this supernatant and the heating and centrifugation were repeated. The supernatant from this centrifugation was brought to 0·1M-NaCl with 3M-NaCl and 2·5vol. of ethanol was added with constant shaking. This ethanolic solution was held at −10° for 1hr. and was then centrifuged at 35000*g* for 10min. The resulting precipitate was taken up in 5ml. of 3mM-EDTA (pH 5·2) and 2·5ml. of 6M-potassium acetate was added, followed by dropwise addition of ethanol to final concentration 20%. After remaining at −10° for 1hr., the mixture was centrifuged at 1500*g* for 15min. The precipitate was again taken up in 5ml. of 3mM-EDTA (pH 5·2) and reprecipitated with potassium acetate and ethanol. This precipitate was suspended in 4ml. of distilled water. The suspension was clarified by centrifugation at 1500*g* for 10min. The supernatant was brought to 0·1M-NaCl and 2·5vol. of ethanol was added. The flocculent RNA precipitate was recovered by centrifugation and dried *in vacuo*. This preparation was dissolved in 1ml. of distilled water and portions were taken for the determination of DNA (Burton, 1956), protein (Lowry, Rosebrough, Farr & Randall, 1951) and RNA. RNA was estimated from its extinction at 260mμ; 100μg. of RNA/ml. had E 2·5.

Ribosomal RNA was prepared by the method of Di Girolamo, Henshaw & Hiatt (1964). Tobacco-mosaic-virus RNA was a gift of Dr S. G. Wildman, University of California, Los Angeles.

Sucrose-density-gradient studies. Linear sucrose density gradients were prepared in buffer containing 0·01M-sodium acetate, 3mM-EDTA and 0·1M-NaCl, pH 5·2. RNA (0·3–1·0mg. in 0·8ml. of buffer) was carefully layered on 28ml. of the gradient and centrifuged for 18hr. at 70000*g* in the SW 25 rotor of the Spinco preparative ultracentrifuge. The bottom of the tube was punctured and 0·8ml. (7-drop) fractions were collected for determination of their extinction at 260mμ. The individual fractions were then combined into five or six groups; 2·5vol. of ethanol was added to each to precipitate the RNA. These combined fractions were held at −10° for 1hr. and then centrifuged at 1500*g* for 15min. The resulting RNA precipitates were dried *in vacuo*, taken up in 0·2–0·3ml. of distilled water, and frozen.

Base analyses. Base compositions of purified RNA samples were determined by the method of Katz & Comb (1963). Hydrolysis was carried out in 0·3N-KOH for 20hr. at 25° and the neutralized hydrolysate was chromatographed on Dowex 50 resin (X4; H^+ form; 200–400 mesh). The eluted nucleotides were assayed by their extinctions at optimum wavelengths, without prior resolution of AMP and CMP.

Determination of messenger RNA activity. The ability of the RNA preparations to stimulate the incorporation of labelled amino acids into a cell-free system of *E. coli* was taken as a routine measure of their messenger RNA content. *E. coli* strain number AB 259 (B_1^-) Zam (obtained from Dr Patrice Zamenhof, University of California, Los Angeles) was grown at 37° in a bacterial shaker in the following medium (amounts are in g./l.): NaOH 0·04, NaCl 5, tryptone 10, glucose 20, yeast extract 5, K_2HPO_4 11, KH_2PO_4 8·5. When the culture reached a growth density of 1·8g./l., it was chilled and the bacteria were obtained by centrifugation. The washed cells were ground with alumina and a cell-free extract was prepared. This extract was incubated at 37° for 80min. to reduce the concentration of endogenous messenger RNA, then dialysed, divided into portions, and stored at −60° as described by Nirenberg (1963).

Messenger RNA activity was assayed by the method of Nirenberg (1963), by using a reaction volume of 0·25ml. which contained 1·17mg. of the preincubated *E. coli* system (S-30 fraction). RNA preparations were added dissolved in distilled water. After incubation at 37°, the reaction was stopped by addition of 2ml. of 5% trichloroacetic acid containing 1% of the appropriate unlabelled amino acid. This mixture was placed in a water bath at 90° for 20min. to hydrolyse aminoacyl-transfer RNA. The protein precipitates were then dispersed and the suspensions were filtered under vacuum through a Millipore filter (25mm. diam., 0·8μ pore size). Each precipitate was washed with three 10ml. portions of cold 5% trichloroacetic acid, three 10ml. portions of chloroform–ethanol (1:1), and two 10ml. portions of chloroform. The filter containing the air-dried precipitate was placed in 5ml. of a scintillator system in toluene, composed of 0·5% 2-phenyl-5(-biphenyl-2-yl)-1,3,4-oxadiazole and 0·01% 1,4-bis-(5-phenyloxazol-2-yl)-benzene. Radioactivity was measured in the Packard Tri-Carb spectrometer at an efficiency of 53·5%. All assays were performed in duplicate or triplicate.

Cerebral and hepatic ribosomes and pH 5 fractions were prepared from rat tissues by the method of Zomzely, Roberts & Rapaport (1964) and were stored as frozen pellets at −60°.

Characteristics of the E. coli *cell-free system.* Incorporation of [^{14}C]phenylalanine into protein by the preincubated S-30 preparation from *E. coli* was strikingly stimulated by

Table 1. *Stimulation of amino acid incorporation into protein of* Escherichia oli *S-30 fraction in the presence of various additions*

The reaction mixture (0·25ml.) contained 100mM-tris-HCl (pH 7·8), 50mM-KCl, 12mM-magnesium acetate, 1mM-NaATP, 0·015mM-GTP (sodium salt), 10mM-creatine phosphate (sodium compound), 6mM-mercaptoethanol, 0·12mg. of creatine phosphokinase, 0·1mM concentrations of each of 20 L-amino acids except phenylalanine, 0·25μc of L-[U-^{14}C]phenylalanine (367mc/m-mole) and 1·17mg. of S-30 protein. Incubation was carried out for 40min. at 37°.

Addition	Amino acid incorporation (counts/min./mg. of protein)
None	1213
None, deproteinized at zero time	103
Tobacco-mosaic-virus RNA (37·5μg.)	18500
Polyuridylic acid (50μg.)	90400
Ribonuclease (1μg.)	117

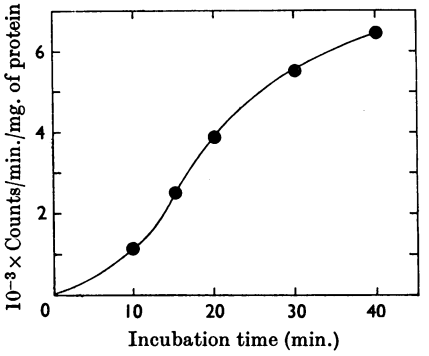

Fig. 1. Stimulation of amino acid incorporation in the *E. coli* S-30 system by tobacco-mosaic-virus RNA. The basic composition of the reaction mixture is given in Table 1. In addition, the reaction mixture (0·25 ml.) contained 60 μg. of tobacco-mosaic-virus RNA.

the addition of high-molecular-weight tobacco-mosaic-virus RNA and by polyuridylic acid (Table 1). The latter (50 μg.) produced a stimulation greater than 70-fold. The reaction was completely inhibited by 1 μg. of ribonuclease. The time-course of the incorporation of [^{14}C]phenylalanine into protein in the presence of tobacco-mosaic-virus RNA, after a lag period, proceeded linearly for approx. 20 min., after which the rate gradually fell (Fig. 1). These data revealed that the *E. coli* amino acid-incorporating system employed was very similar to that originally described by Nirenberg & Matthaei (1961), with extracts prepared from *E. coli* strain W3100. It therefore seemed suitable for the assay of messenger activity in RNA preparations.

RESULTS

Properties of RNA isolated from mammalian nuclear and ribosomal preparations. In the present investigations, the concentration of RNA in the crude nuclear preparations varied from 79 to 84% of the combined RNA, DNA and protein. The concentration of DNA varied from 6 to 15% and that of protein from 7 to 13%. The yield of the cerebral nuclear RNA was between 45 and 63 μg. of RNA/g. of rat brain or about 2·5–3·5% of the total cerebral RNA. The yield of the hepatic nuclear RNA was 280 μg./g. of liver (4·5% of total hepatic RNA). The recovery of RNA from the crude nuclear pellets was approx. 21% for the cerebral preparation and 34% for the hepatic preparation.

Cerebral RNA isolated from the crude nuclear preparation had a characteristic base composition which was considerably higher in UMP and lower in CMP than ribosomal RNA (Table 2). These values are in agreement with those reported by Egyhazi & Hydén (1966*b*) for the base compositions of the RNA fractions in neurons and glia that are most readily degraded by ribonuclease and are therefore presumably single-stranded. The large differences between AMP and UMP, and between GMP and CMP concentrations of the cerebral nuclear RNA preparation, also suggested the presence of a considerable proportion of single-stranded RNA (Houssais & Attardi, 1966).

For comparison, values are given for the base composition of homologous liver DNA as reported by Wyatt (1951). These values may also be assumed to apply to rat cerebral DNA. McCarthy & Hoyer (1964) have shown identity of polynucleotide sequences of DNA prepared from different tissues of the mouse. The high dTMP content and low dCMP content of the DNA appeared to parallel the values for UMP and CMP respectively in the cerebral nuclear RNA preparation. However, the dGMP and dAMP concentrations of the DNA showed no similarity to the GMP and AMP concentrations of this cerebral nuclear RNA. Furthermore, the cerebral nuclear RNA preparation did not exhibit a base composition intermediate in value between that of DNA and ribosomal RNA. It is likely that a significant portion of RNA in the crude nuclear preparation from rat brain had a base composition that was different from that of ribosomal RNA or of the total cell DNA. However, the (GMP + CMP)/(AMP + UMP) ratios of this cerebral nuclear RNA (1·35–1·42) were closer than those of cerebral ribosomal RNA (1·58 and 1·62) to the (dGMP + dCMP)/(dAMP + dTMP) ratio of homologous DNA (0·75). This finding implied that the degree of complementarity to total cell DNA was greater for the cerebral nuclear RNA preparation than for cerebral ribosomal RNA. Mach & Vassalli (1965*b*), working with rapidly labelled RNA of spleen and lymph nodes, obtained a base ratio 1·34, which was stated to be compatible with a mixture of 65–70% ribosomal RNA and 30–35% DNA-like RNA. The nucleotide base ratios for the cerebral nuclear RNA in the present investigations suggested that this preparation may be contaminated with significant quantities of cytoplasmic ribosomal RNA. However, the base composition of purified mammalian nuclear RNA has not been sufficiently well established to permit accurate estimation of this contamination from measurements of base ratios.

In contrast with the situation with the cerebral nuclear RNA preparation, the CMP content of RNA isolated from crude hepatic nuclear preparations was only slightly below that of hepatic ribosomal RNA and the UMP contents were not significantly different. The overall base composition of this hepatic nuclear RNA thus bore a closer resemblance to ribosomal RNA than did the cerebral nuclear RNA. It is possible that the hepatic nuclear fraction before extraction was

Table 2. *Base composition of RNA isolated from nuclear and ribosomal preparations of rat brain and liver*

Nucleotides in the RNA hydrolysates were estimated spectrophotometrically after chromatography (Katz & Comb, 1963). Absorption maxima for nucleotides in 0·05 N-HCl were found to be: UMP, 260 mμ; GMP, 257 mμ; CMP, 279 mμ; AMP, 257 mμ.

Preparation	Nucleotide composition (%)			
	AMP	GMP	CMP	UMP
Cerebral nuclear RNA	17·1	35·0	23·6	24·3
	15·3	35·9	22·3	26·6
	16·2	35·0	22·5	26·3
Cerebral ribosomal RNA	17·2	33·2	28·0	21·5
	16·8	34·5	27·2	21·4
Liver nuclear RNA	16·1	36·6	25·9	21·5
	19·3	35·1	24·6	21·0
Liver ribosomal RNA	17·6	34·6	29·1	18·9
	17·9	32·7	28·5	20·8
	dAMP	dGMP	dCMP	dTMP
Rat liver DNA (Wyatt, 1951)	28·6	21·4	21·5	28·4

contaminated with ribosomes to a greater extent than the corresponding cerebral fraction. In this connexion, preparations of hepatic nuclear RNA have previously been described with lower (GMP + CMP)/(AMP + UMP) ratios and, presumably, of greater purity than those described here (Maggio, Siekevitz & Palade, 1963; Steele, Okamura & Busch, 1965).

Sucrose-density-gradient analyses of the nuclear RNA preparations from cerebral and hepatic tissue revealed two major peaks with sedimentation values (18s and 28s) similar to those of the two major species of mammalian ribosomal RNA (Fig. 2). However, the breadths of the peaks and the considerable amounts of RNA sedimenting more rapidly than the heavier peaks suggested that the RNA species isolated from the crude nuclear preparations were more heterogeneous than the corresponding ribosomal RNA species. Cerebral and hepatic ribosomal RNA each sedimented as two well-defined peaks in the presence of EDTA (D. M. Schneider, unpublished work). There was only a small peak in the 4s region of the cerebral and hepatic nuclear RNA preparations, indicating that very little transfer RNA was present in either case. Density-gradient analysis of the cerebral nuclear RNA uniformly revealed a peak in the 18s region that was higher than the peak in the 28s region. The opposite situation held for the hepatic nuclear RNA preparations, where the 28s peak was predominant (a result also obtained by Hiatt, 1962; Samarina, 1964; Steele *et al.* 1965).

Only a small amount of material absorbing at 260 mμ was present in the region close to the top of the gradient in either cerebral or hepatic RNA preparations. This finding indicated that low-molecular-weight materials such as oligonucleotides or oligodeoxynucleotides were virtually absent. These nucleotides have been shown to inhibit nuclear RNA stimulation of amino acid incorporation in the *E. coli* system (Di Girolamo *et al.* 1964), thereby interfering with assays of messenger RNA activity. They were present in large amounts in a previously reported preparation of cerebral nuclear RNA (Zemp, Wilson, Schlesinger, Boggan & Glassman, 1966).

Messenger activity of nuclear RNA preparations. Unfractionated nuclear RNA preparations from either brain or liver were capable of enhancing the incorporation of phenylalanine into protein in the *E. coli* S-30 system (Table 3). However, the cerebral nuclear RNA preparation was consistently more active than the corresponding hepatic preparation. The cerebral nuclear RNA preparation also stimulated the incorporation of radioactive leucine and lysine in this system (Table 3). This result implies that the incorporation represented true protein synthesis. The stimulatory capacity of the cerebral nuclear RNA preparation was 12–27 times that of RNA derived from cerebral ribosomes, but only about 15% of that observed with tobacco-mosaic-virus RNA (see Table 1). Viral RNA is a homogeneous high-molecular-weight material which is largely single-stranded and has very high template activity (Markham, 1963). The stimulation of amino acid incorporation was proportional to the amount of the cerebral nuclear RNA preparation added, up to a concentration of about 600 μg. of RNA/ml. (Fig. 3). The system was not saturated by RNA concentration over 1 mg. of RNA/ml. These data are similar to those obtained earlier by Kenney & Kull (1963) with a hepatic nuclear RNA preparation.

RNA was recovered from the sucrose density

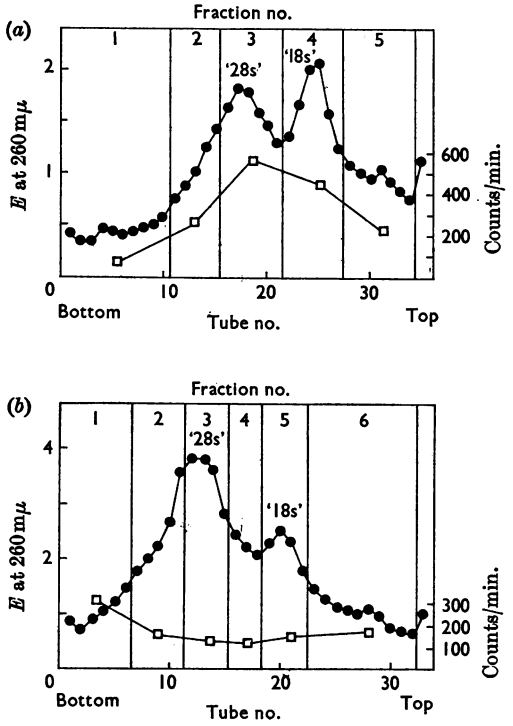

Fig. 2. Sucrose-density-gradient profiles and amino acid-incorporating activities of RNA isolated from crude nuclear preparations of (*a*) rat brain and (*b*) rat liver. The RNA preparation (0·3–1·0 mg.) was dissolved in 0·8 ml. of medium composed of 0·01 M-sodium acetate, 0·1 M-NaCl and 3 mM-EDTA (pH 5·2) and was layered on a linear sucrose gradient (5–20%) containing the same materials. The gradient was then centrifuged at 22 000 rev./min. for 18 hr. The fractions from the gradient were collected in tubes for determination of extinction at 260 mμ. The contents of the tubes were pooled as indicated by the vertical lines. These combined fractions were then precipitated by the addition of 2·5 vol. of ethanol, dried and assayed for capacity to stimulate amino acid incorporation in the *E. coli* system. Incubation conditions are given in Table 1. The reaction mixture (0·25 ml.) contained 1·17 mg. of *E. coli* S-30 protein and 0·25 μc of L-[U-^{14}C]phenylalanine (392 mc/m-mole). Incubation was for 40 min. ●, *E* 260 mμ; □, counts/min./100 μg. of RNA for each fraction.

gradients by precipitation with ethanol and pooled into five or six fractions as indicated in Fig. 2. These fractions were then assayed for stimulatory activity in the amino acid-incorporating system from *E. coli*. The assay was carried out at concentrations of RNA at which a linear relation to stimulation existed. The messenger activities of cerebral and hepatic RNA species were found to be widely distributed throughout the gradients. The most active fraction of liver sedimented more

Table 3. *Stimulation of the* Escherichia coli (*S*-30 *fraction*) *amino acid-incorporating system by cerebral and hepatic RNA preparations*

The composition of the reaction mixture is given in Table 1. The mixture of 20 L-amino acids added included all amino acids except the ^{14}C-labelled amino acid. The reaction mixture (0·25 ml.) contained 1·17 mg. of S-30 protein and was incubated for 40 min. in each instance.

Addition	Amino acid incorporation (counts/min./mg. of protein)
0·25 μc of L-[U-^{14}C]phenylalanine (392 mc/m-mole)	
None	1480
Cerebral nuclear RNA (51 μg.)	4880
Cerebral ribosomal RNA (68 μg.)	1842
Hepatic nuclear RNA (68 μg.)	3240
0·25 μc of L-[U-^{14}C]lysine (247 mc/m-mole)	
None	885
Cerebral nuclear RNA (51 μg.)	1723
0·05 μc of L-[U-^{14}C]leucine (280 mc/m-mole)	
None	1027
Cerebral nuclear RNA (51 μg.)	1920

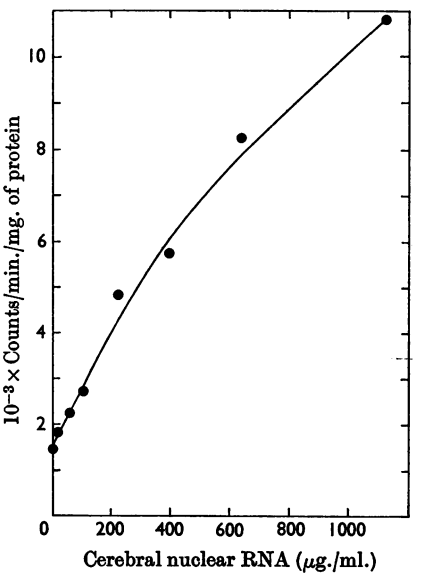

Fig. 3. Influence of varying concentrations of a cerebral nuclear RNA preparation on amino acid-incorporating activity of the *E. coli* system. The composition of the reaction mixture is given in Table 1. The reaction mixture (0·25 ml.) contained 1·17 mg. of S-30 protein and 0·25 μc of L-[U-^{14}C]phenylalanine (392 mc/m-mole). Incubation was carried out for 40 min.

rapidly than the cerebral fraction that possessed maximal activity (Fig. 2). Barondes, Dingman & Sporn (1962) found the most active fraction of a

hepatic nuclear RNA preparation to be in a region greater than 40s.

Stimulation of amino acid incorporation in rat cerebral ribosomes. Nuclear RNA preparations with demonstrated stimulatory activity on amino acid incorporation in the *E. coli* S-30 system were also assayed in ribosomal systems prepared from rat liver and cerebral cortex. The complete cerebral system, which included the pH 5 fraction or the post-microsomal supernatant, responded to nuclear RNA preparations with only a small increase in amino acid incorporation into ribosomal protein (see, for example, Table 4). As expected, omission of these sources of activating enzymes markedly reduced basal values of amino acid incorporation. Addition of hepatic or cerebral nuclear RNA preparations strikingly enhanced amino acid incorporation in the cerebral system which was deficient in activating enzymes, but was essentially without effect on amino acid incorporation in the corresponding hepatic system. Thus in this cerebral system, the cerebral nuclear RNA preparation (51 μg.) elicited a twofold increase in leucine incorporation into ribosomal protein (Table 4). Addition of the hepatic nuclear RNA preparation (82 μg.) resulted in a 50% stimulation. The activities of these nuclear RNA preparations were not due to contamination with transfer RNA, since the addition of a mixture of purified transfer RNA species from *E. coli* did not eliminate the response.

Table 4. *Incorporation of leucine into protein by cerebral ribosomes*

The reaction mixture was similar to that described in Table 1. However, the mixture (0·25 ml.) contained 0·25 μc of L-[U-^{14}C]leucine (170 mc/m-mole) and 117–179 μg. of cerebral ribosomes in place of S-30 protein. Incubation was carried out for 40 min. A mixture of transfer RNA species (*E. coli* B) was obtained from Schwarz BioResearch Inc.

Addition	Amino acid incorporation counts/min./mg. of protein)
None	4190
135 μg. of pH 5 fraction	14800
135 μg. of pH 5 fraction + 51 μg. of cerebral nuclear RNA	16000
51 μg. of cerebral nuclear RNA	8400
82 μg. of hepatic nuclear RNA	6120
50 μg. of transfer RNA (*E. coli B*)	5210
50 μg. of transfer RNA (*E. coli B*) + 51 μg. of cerebral nuclear RNA	8620

DISCUSSION

The present investigations demonstrate the occurrence of messenger activity in RNA preparations from cerebral tissue. The active RNA was isolated from crude nuclei prepared from rat brain and was capable of stimulating the incorporation of amino acids into proteins of *E. coli* and cerebral ribosomal systems. One earlier report has appeared describing template activity of similar magnitude for a nuclear RNA preparation from mouse brain assayed in the presence of homologous ribosomes (Herriman & Hunter, 1965). Messenger activity has also been reported in various RNA preparations from other mammalian sources employing the *E. coli* system, which is highly sensitive to exogenous messenger RNA (Nirenberg, 1963). These other sources include liver (Barondes *et al.* 1962; Brawerman, Gold & Eisenstadt, 1963; Di Girolamo *et al.* 1964; Hadjiolov, 1966), spleen and lymph nodes (Mach & Vassalli, 1965*a*), placenta (Silverstein & Bondy, 1966), prostate gland (Liao, 1965), thyroid gland (Cartouzou, Manté & Lissitzky, 1965) and amniotic cells (Yoshikawa-Fukada, 1966). In addition, studies have demonstrated stimulation of amino acid incorporation into homologous ribosomes by hepatic nuclear RNA preparations (Korner & Munro, 1963; Kenney & Kull, 1963; Decken, 1965; Dukes, Sekeris & Schmid, 1966), prostatic nuclear RNA (Liao, 1965) and reticulocyte RNA (Arnstein, Cox & Hunt, 1964). In the experiments reported here, the ability of cerebral RNA to stimulate amino acid incorporation in the cerebral ribosomal system was most readily demonstrated in the absence of the pH 5 fraction or the post-microsomal supernatant from cerebral tissue. These materials are normally employed as sources of amino acid-activating enzymes (see Hoagland, Keller & Zamecnik, 1956). Stimulatory activity of the cerebral nuclear RNA preparation could not be demonstrated in an hepatic ribosomal system even in the absence of the hepatic pH 5 fraction. The component in crude preparations of cerebral and hepatic activating enzymes and in hepatic ribosomes which inhibited this response may be ribonuclease (C. E. Zomzely, S. Roberts & C. Provost, unpublished work), which would be expected to degrade template RNA quite readily (Bautz, 1963). Single-stranded RNA with molecular weight below 20000 has virtually no messenger activity (Martin & Ames, 1962). Only one internucleotide linkage in 50 would have to be ruptured to reduce the molecular weight of an RNA molecule from 1 million to fragments with an average molecular weight of 20000. Template RNA may be better preserved in the *E. coli* system where the ribosomes absorb and inhibit ribonuclease (Wade & Robinson, 1965).

Sucrose-density-gradient analyses revealed that cerebral RNA isolated from crude nuclear preparations was heterogeneous. Template activity was widely distributed throughout the gradient. This

finding was consistent with the observations of Herriman & Hunter (1965) on the stimulatory capacity of fractions obtained after chromatography of a cerebral nuclear RNA preparation on a methylated serum albumin–silicic acid column. In the present investigations, the base composition of the cerebral nuclear RNA preparation was not identical with that of homologous DNA, but resemblances were noted. Similar observations have been made with nuclear RNA preparations from other mammalian tissues (Sibatani, de Kloet, Allfrey & Mirsky, 1962; Maggio *et al.* 1963; Mach & Vassalli, 1965*a*; Steele *et al.* 1965). Close similarity between the base composition of nuclear RNA and DNA cannot be expected in mammalian tissues because this property of nuclear RNA varies from one chromosome to another (Edstrom & Beerman, 1962) and only a fraction of the total genetic complement is expressed by each cell. Moreover, messenger RNA is complementary to only one-half of double-stranded DNA (McCarthy & Bolton, 1964). The present data on base composition of the cerebral nuclear RNA preparation revealed the occurrence of a considerable proportion of non-ribosomal RNA. The latter may include, in addition to messenger RNA, precursors of ribosomal and transfer RNA as well as homopolymers such as polyadenylic acid (Chambon, Weill & Mandel, 1963; Hadjivassiliou & Brawerman, 1966). Unequivocal identification of these various species requires their prior purification. The heterogeneity observed in sucrose-density-gradient analyses of RNA from crude nuclear preparations suggested that separation on a basis of molecular size was incomplete. Considerable messenger activity was present in RNA molecules exhibiting sedimentation velocities similar to those of ribosomal RNA. In fact, preponderance of the latter may mask the contribution made to the extinction profile by the actual messenger moieties. As an approach to circumventing this difficulty, pulse-labelling with [^{32}P]phosphate has been employed to deduce the base composition of the most rapidly labelled RNA of cerebral nuclear preparations (Jacob *et al.* 1966). However, use of this procedure assumes that there is uniform labelling of phosphate within the RNA molecules. This assumption seems to be unwarranted (Spencer, 1962).

In the present investigations, RNA isolated from crude hepatic nuclear preparations appeared to have a higher content of ribosomal RNA than RNA from crude cerebral nuclear preparations. Thus the base composition and the sucrose-density-gradient profile of the hepatic nuclear RNA preparation were quite similar to the same characteristics of ribosomal RNA. As noted above, this was not the case with the cerebral nuclear RNA preparation. The indication of a higher content of ribosomal RNA in the hepatic preparation was supported by the finding that the stimulatory activity of the hepatic nuclear RNA in the *E. coli* system was lower than that of the cerebral nuclear RNA. This was true of both the unfractionated preparations and fractions obtained from the sucrose density gradients. Since the same procedures were employed for the isolation of cerebral and hepatic nuclei and for the extraction of RNA from these sources, it is possible that the differences noted in the two preparations reflected variations in their properties in the natural state. However, these differences could also have been due to a greater degree of cytoplasmic contamination in the hepatic nuclear preparation than in the cerebral nuclear preparation from which the RNA species were isolated.

From the sedimentation values of cerebral nuclear RNA fractions with messenger activity, and on the basis of a triplet code, it can be estimated that the synthesis of a range of proteins with molecular weights varying from 4000 to over 140000 could be directed. However, cerebral messenger RNA species may be polycistronic and break down into smaller units before transfer to the cytoplasm (see, e.g., Samarina, 1964; Mach & Vassalli, 1965*b*), thereby coding for proteins of lower molecular weights than those calculated here.

Note added in proof. Evidence has been obtained in support of the conclusion that the cerebral nuclear RNA preparation selectively stimulated the synthesis of specific peptides in the cerebral ribosomal system lacking pH 5 enzymes. Incubation was carried out with a mixture of 14 ^{14}C-labelled amino acids (Schwartz Bioresearch Inc.) and the specific activities of the amino acids released by hydrolysis of the ribosomal protein were measured. Stimulation of incorporation by cerebral nuclear RNA varied from 0% (tyrosine, glutamic acid) to 80% (leucine, arginine), indicating that the new peptides synthesized differed in amino acid composition from those formed endogenously.

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