the losses were nearly identical for both preparations.

The rate of cleavage of galactose from the stored mucopolysaccharide was tenfold lower in generalized gangliosidosis; after 18 hours, 38.9 percent of the galactose in the mucopolysaccharide was released by the preparation from a normal patient, whereas 3.8 percent was released by the preparation from patients with generalized gangliosidosis (Fig. 1). The same result was obtained in a second patient with the disease.

One objection to the use of partially purified β-galactosidase preparations is that the enzyme may not fractionate the same in diseased tissue. This objection is removed by our previous demonstration (*4*) with whole liver homogenates that β-galactosidase activity (both for *p*-nitrophenyl-β-D-galactopyranoside and for ganglioside GM_1) is 20- to 30-fold lower than normal in this disease. Our previous work (*4*) also demonstrated that inhibitors of β-galactosidase activity (for *p*-nitrophenyl-β-D-galactopyranoside and for ganglioside GM_1) are not responsible for the lowered enzymic activity. We have assumed here that endogenous inhibitors do not account for the impaired cleavage of galactose when the mucopolysaccharide serves as enzyme substrate.

In another experiment we measured the rate of cleavage of galactose from sialic acid–free fetuin. This glycoprotein contains an oligosaccharide chain with a terminal galactose linked to hexosamine, and a sialic acid moiety linked to the galactose. Sialic acid–free fetuin was prepared by mild acid hydrolysis (*13*) to give a glycoprotein with a free terminal galactose. This glycoprotein (2 mg) was then incubated with purified preparations of β-galactosidase obtained from normal (two patients) and generalized gangliosidosis (two patients) livers. The preparations from normal liver liberated small (average, 5.5 μg) but readily detectable amounts of galactose after 18 hours of incubation at 37°C, whereas no detectable galactose was liberated by the generalized gangliosidosis preparation.

Our results demonstrate an impaired cleavage of galactose from a mucopolysaccharide in generalized gangliosidosis. It appears likely that the normal degradation of these and similar macromolecules containing galactose involves the participation of lysosomal β-galactosidase and that their accumulation is the result of a block in their catabolism. The bony abnormalities so characteristic of generalized gangliosidosis may be explained by defective catabolism of mucopolysaccharides in connective tissue.

MONICA C. MACBRINN
SHINTARO OKADA, MAE WAN HO
CHIN CHAI HU, JOHN S. O'BRIEN
Department of Neurosciences, University of California at San Diego, La Jolla 92037

References and Notes

1. J. S. O'Brien, M. B. Stern, B. H. Landing, J. K. O'Brien, G. N. Donnell, *Amer. J. Dis. Child.* **109**, 338 (1965).
2. N. K. Gonatas and J. Gonatas, *J. Neuropathol. Exp. Neurol.* **24**, 318 (1965).
3. B. H. Landing, F. N. Silverman, J. M. Craig, M. D. Jakoby, M. E. Lahey, D. L. Chadwick, *Amer. J. Dis. Child.* **108**, 503 (1964).
4. S. Okada and J. S. O'Brien, *Science* **160**, 1002 (1968).
5. R. M. Norman, H. Urich, A. Tingey, R. A. Goodbody. *J. Neuropathol. Exp. Neurol.* **78**, 409 (1959); E. Farkas-Bargeton, *Proc. Int. Congr. Neuropathol. 5th* (1965), p. 135; C. R. Scott, D. Lagunoff, B. F. Trump, *J. Pediat.* **71**, 357 (1967).
6. K. Suzuki, *Science* **159**, 1471 (1968).
7. S. Gatt, *Biochim. Biophys. Acta* **139**, 192 (1967).
8. H. Roth, S. Segal, D. Bertoli, *Anal. Biochem.* **10**, 32 (1965).
9. N. F. Boas, *J. Biol. Chem.* **204**, 553 (1953).
10. K. Suzuki and G. C. Chen, *J. Lipid Res.* **8**, 105 (1967).
11. F. S. Wusteman, A. G. Lloyd, K. S. Dodgson, *J. Chromatogr.* **21**, 32 (1966).
12. S. Gatt and M. M. Rapport, *Biochim. Biophys. Acta* **113**, 567 (1966).
13. R. G. Spiro, *Meth. Enzymol.* **8**, 3 (1966).
14. Supported in part by grant NB06576 from the National Institute of Neurological Diseases and Blindness.

14 November 1968; revised 8 January 1969

N-Formylseryl-Transfer RNA

Abstract. *The reactions between serine and transfer RNA from baker's yeast and from* Escherichia coli *have been investigated. Results obtained from in vitro, in vivo, and chemical syntheses and from electrophoretic, chromatographic, and radioautographic analyses demonstrate that* N*-formylseryl-transfer RNA is formed in these systems.*

Formation of *N*-formylmethionyl-tRNA in *Escherichia coli* and in yeast was reported by Marcker and Sanger (*1*), and the importance of this particular transfer RNA (tRNA) derivative on the mechanism of protein synthesis has been elucidated (*2*). I now present evidence of the formation of *N*-formylseryl-tRNA.

The tRNA's of baker's yeast and *E. coli* B, ^{14}C-serine, ^{35}S-methionine, ^{14}C-formate, and ^{14}C-adenosine triphosphate (ATP) were purchased (Schwarz BioResearch, Inc.) and stored at −20°C. Occasionally tRNA's were prepared from yeast according to the method of Zubay (*3*). The purchased tRNA's gave a single absorption peak when chromatographed in water on a Sephadex G-25 column, but some nuclease was present; and in the ribonuclease or alkaline hydrolyzates of both tRNA's fewer than 60 percent of the terminal groups were adenosine while more than 40 percent were cytosine, a fact that has been reported (*4*). Accordingly, in critical experiments the commercial tRNA's were treated with phenol, extracted in aqueous phase, washed twice with peroxide-free ether, dried, and recharged with adenosine monophosphate (*5*). Radioactive chemicals were generally purified with ion-exchange resins and subsequent electrophoresis or chromatography until they gave a single band on the radioautograph. Strains of *E. coli* B and A19 were

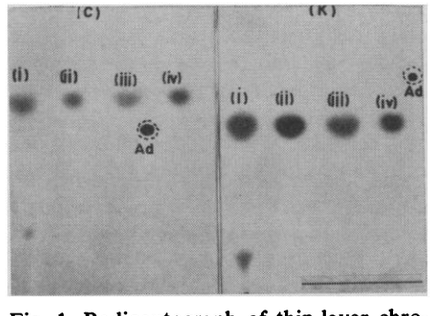

Fig. 1. Radioautograph of thin-layer chromatography of *N*-formylseryl-adenosine isolated from ribonuclease digests of tRNA of various treatments on two plates, cellulose (*C*) and kieselgel (*K*). Compounds were isolated as B bands of electrophoretogram and purified by passing them through Dowex I. They were isolated from (i) yeast tRNA charged with ^{14}C-serine in vivo, (ii) and in vitro, (iii) yeast tRNA terminally labeled with ^{14}C-adenosine and subsequently charged with unlabeled serine, (iv) *N*-^{14}C-formylseryl-tRNA synthesis in the manner described in the text. *Ad*, position of unlabeled adenosine located under ultraviolet light. The R_F values were as follows: on cellulose plate: adenosine, 0.62; *N*-formyl compounds, 0.70; kieselgel: adenosine, 0.86; *N*-formyl compounds, 0.60. Scale, 5 cm.

cultured and *S*100 enzymes from the 10^5g fraction (*6*) from both organisms were prepared by the method of Nirenberg and Matthaei, except that the fresh *S*100 proteins were immediately passed through a G-25 Sephadex column previously equilibrated with standard buffer (*6*). The fractions from the first main absorption peak were pooled and reagent grade glycerol (*7*) was added to 50 percent (by volume); this pooled material was stored at −20°C where the enzyme remained unfrozen, and its activity was undiminished for more than 6 months. The yield of seryl-tRNA synthetase varied, but, on the average, the specific activity of the enzyme extracted from yeast was approximately 10 to 15 units per milligram of protein (*8*), whereas that from *E. coli* amounted to about 19 to 22 units. Here, a unit of enzyme catalyzes the formation of 1 nmole of aminoacyl-tRNA in 10 minutes at 37°C.

N-Acetyl-^{14}C-serine and *N*-acetyl-^{35}S-methionine were synthesized, according to the method of Kolb and Toennies (*9*) and *N*-formyl-^{14}C-serine and *N*-formyl-^{35}S-methionine were synthesized by the method of Sheehan and Yang (*10*). Products were isolated by chromatography on a Dowex-50 (H^+ form, 200 mesh, X8) column (1 by 2 cm) and further purified by paper chromatography or electrophoresis in combination with radioautography. Formylation of ^{12}C-seryl-tRNA with ^{14}C-formate was carried out by a slightly modified method of Marcker and Sanger (*1*). Terminally labeled ^{14}C-adenosine tRNA was prepared according to the procedures of Bretscher (*11*). Thin-layer chromatography (TLC) plates (10 by 20 cm) of kieselgel and cellulose were purchased from Merck Co., Darmstadt, Germany, and chromatographs were developed in a tank containing a mixture of isopropanol, concentrated NH_4OH, and H_2O (7:1:2 by volume). Filter paper disks (22 mm diameter) as well as papers for electrophoresis were MN 218 (*12*). For radioautography an x-ray film "Royal Blue" (20 by 40 cm) (Eastman Kodak) was used.

For routine assay of the formation of seryl-tRNA, 0.05 to 0.1 ml of the reaction mixture was incubated for 5 to 10 minutes at 37°C. The mixture (*p*H 7.5) contained (μmole/0.1 ml): tris-HCl, 10; ATP, 1.25; $MgCl_2$, 0.5 to 1.5; EDTA, 0.25 plus tRNA, 0.04 to 0.5 mg; ^{14}C-serine or ^{35}S-methionine, 0.05 μc; enzyme, 0.01 to 0.15 unit. Duplicate series of samples and blanks (containing no enzyme) were run each time. The reaction was stopped by placing the test tubes in an ice bath and immediately adding 20 μl of 1*N* HCl. Samples (50 μl) from each tube were taken in duplicates, placed on a filter paper disk, washed, dried, mounted, and counted in a scintillation apparatus (*13*). For the preparation of large amounts of "charged" tRNA, 1- to 2-ml reaction mixtures were used. For the preparation of seryl-tRNA, the amount of enzyme was restricted to approximately 0.1 unit/ml, incubation time was limited to 5 minutes, and Mg^{++} ion concentration was maintained at about *3* m*M*. For the synthesis of *N*-formylseryl-tRNA, larger doses of enzyme (1.0 to 1.5 unit/ml), longer time of incubation (10 to 15 minutes), and higher concentration of Mg^{++} (15 m*M*) were necessary.

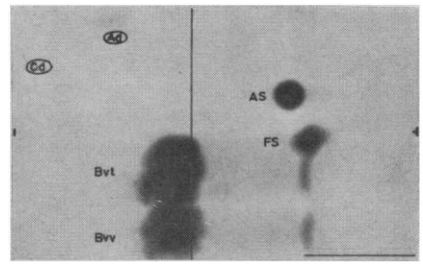

Fig. 2. Radioautograph of electrophoretogram of partial alkaline hydrolyzates of B band prepared from yeast tRNA in vitro (*Bvt*) and in vivo (*Bvv*). They were treated with 2*N* ammonium hydroxide for 10 minutes at room temperature. *Ad*, adenosine under ultraviolet light; *Cd*, cytidine under ultraviolet light; *AS*, *N*-acetyl-^{14}C-serine; *FS*, *N*-formyl-^{14}C-serine. Scale, 5 cm.

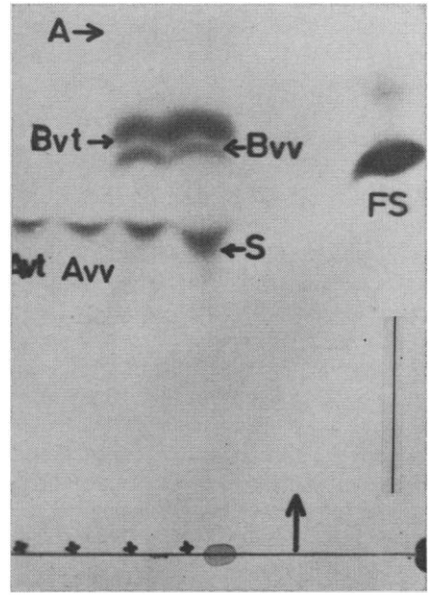

Fig. 3. Radioautograph of TLC (cellulose) of same materials as Fig. 2 and of alkaline hydrolyzates of A band. *S*, free serine; *Avt*, free serine hydrolyzed from A band which had been prepared by in vitro method; *Avv*, same prepared by in vivo method. Scale, 3 cm.

^{14}C-Serine (150 μc, 160 mc/mmole) was incorporated into yeast cells (*1*). From about 2 g of wet yeast, harvested from 250 ml culture, 5.5 mg of RNA (4.8 mc/mmole) was isolated. The RNA was further fractionated on a G-25 Sephadex column and the absorbance (at 260 nm) and radioactivity of fractions were measured. Three absorption peaks were separated, but radioactivity was associated mainly with the first two peaks. The first of the two radioactive peaks contained most of what has been called "B band" material (*1*). It was isolated by electrophoresis and further analyzed (Figs. 1–3).

Initial attempts to isolate tRNA charged with ^{14}C-serine were unsuccessful, only a very small amount of tRNA being precipitated in ethanol. As judged from Sephadex G-25 column chromatography, some 30 percent of input yeast tRNA was degraded after incubation for 20 minutes at 37°C with addition of the *S*100 protein. As much as 50 percent of the amino acid originally bound to tRNA could be isolated from the second Sephadex peak (oligonucleotides) after ribonuclease digest. Therefore tRNA charged with amino acid is quickly hydrolyzed by nuclease present in the crude *S*100 enzyme. Accordingly, the method of isolation of the aminoacyl products was improved.

After incubation of the reaction mixture (5 to 10 minutes at 37°C), a catalytic amount of pancreatic ribonuclease was added, and incubation was continued for 30 minutes more. This was then deproteinated with cold phenol; the aqueous phase was washed with ether twice and passed through a Dowex I (OH^- form, 200 mesh, X8) column (1 by 2 cm) with water to eliminate free amino acids. The eluate containing both seryladenosine and *N*-formylseryladenosine compounds was concentrated and subjected to electrophoresis in 0.05*M* ammonium formate buffer of *p*H 3.5 at 45 volt cm^{-1}. Radioautography after exposure for 4 to 5 days produced two bands at the cathode side of the paper which can be desig-

Table 1. Influence of acyl donors on the synthesis of aminoacyl-tRNA. The reaction mixture (0.1 ml) containing 1.0 mg of yeast tRNA, 0.5 μc of ^{14}C-serine (160 mc/mmole) or ^{14}C-(methyl)-methionine (56.8 mc/mmole), 5 μg of acyl donors, and the other stipulated ingredients were incubated for 10 minutes at 37°C. Yield of radioactivity was measured on the filter paper disk in scintillation vials, and suitable corrections were made (*12*). AcCoA, acetyl coenzyme A; AcP, acetyl phosphate; N^5FTHF, N^5- or N^{10}-formyltetrahydrofolate.

Acyl donor	Aminoacyl-tRNA formed (pmole)	
	Seryl-tRNA	Methionyl-tRNA
None	218.1	747.1
AcCoA	258.7	724.9
AcP	246.5	759.2
N^5FTHF	378.8	866.2
N^{10}FTHF	420.5	1321.5

nated (*1*) as B and A bands according to the proximity to the origin. The two bands were eluted separately for further analyses.

Electrophoretic B bands were isolated from four different sources showing identical migration on two different TLC plates (Fig. 1, *C* and *K*). When B bands from samples prepared in vivo [Fig. 1(i)] and in vitro [Fig. 1(ii)] were partially hydrolyzed in dilute alkali, a component of the hydrolyzate migrated along with *N*-formylserine on electrophoresis (Fig. 2) and on TLC (Fig. 3). When the band matching the position of *N*-formylserine was eluted, hydrolyzed in 1*N* HCl for 30 minutes at 100°C, and subjected to TLC along with ^{14}C-serine standard, serine was the only radioactive spot detected on the radioautograph. Figure 4 shows that both serine and methionine react with either yeast or *E. coli* tRNA, producing *N*-formylaminoacyl-tRNA derivatives. After alkaline hydrolysis, the B band of Fig. 1(iii) yielded radioactive adenosine on TLC plates which coincided with the band of cold adenosine detectable under ultraviolet light. The B band material of Fig. 1(iv) was further hydrolyzed in alkali to demonstrate the presence of formylserine which, after acid hydrolysis, yielded radioactive formate on electrophoresis.

When four different acyl donors were added to the reaction mixture, only the formyl donors, especially the N^{10}-formyltetrahydrofolate (N^{10}FTHF) greatly promoted the synthesis of aminoacyl-tRNA (Table 1).

Evidence provided above suggests that the electrophoretic B band compound is *N*-formylseryladenosine and that *N*-formylseryl-tRNA is formed in vitro in systems of baker's yeast and of *E. coli* as well as in vivo in systems of yeast. Formation of *N*-formylseryladenosine in *E. coli* in vivo has not been investigated.

When band A material was subjected to electrophoresis and TLC after alkaline hydrolysis, only the free amino acid was detected on the radioautogram (Fig. 3). From similar sets of experiments described above, it was identified as seryladenosine, a degradation product of seryl-tRNA. Unlike methionyladenosine, which gives two bands, seryladenosine migrates as one band either on the electrophoretogram or TLC plates (Fig. 4).

No evidence was found that *N*-formylserine or *N*-formylmethionine can be bound directly to yeast tRNA, suggesting that formylation could only be achieved after aminoacyl-tRNA is formed as was noted earlier with *N*-formylmethionyl-tRNA (*1*, *14*).

W. S. KIM

Max-Planck-Institut für Biologie, Abteilung Melchers, 74 Tübingen/W.-Germany

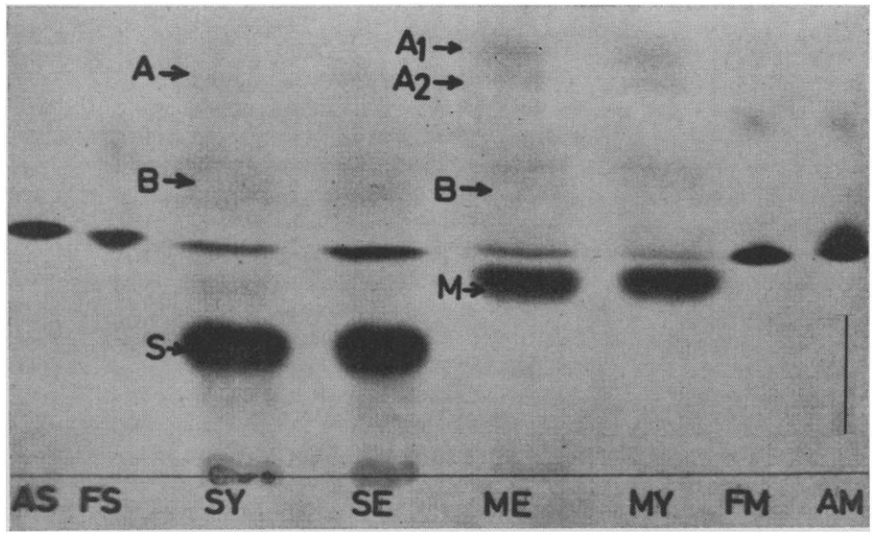

Fig. 4. Radioautograph of TLC of alkaline hydrolyzates of A and B bands on kieselgel plate. The ribonuclease digests of yeast and *Escherichia coli* tRNA's which had been reacted with ^{14}C-serine or ^{35}S-methionine in the reaction mixture containing 0.5 mg of tRNA, 1 μc of radioactive amino acids and other ingredients described in the text, was passed through a Dowex I column with water. This was concentrated, hydrolyzed in 2*N* NH_4OH for 60 minutes at 37°C, dissolved in water, and subjected to TLC on a kieselgel plate for 7 hours at 20°C in a solvent mixture described in the text. *AS*, *N*-acetyl-^{14}C-serine; *FS*, *N*-formyl-^{14}C-serine; *SY*, *SE*, respectively, sample prepared from ^{14}C-serine and yeast tRNA, and that from ^{14}C-serine and *E. coli* tRNA; *ME* and *MY*, sample prepared from ^{35}S-methionine and *E. coli* tRNA and that from ^{35}S-methionine and yeast tRNA; *FM*, *N*-formyl-^{35}S-methionine; *AM*, *N*-acetyl-^{35}S-methionine; *S*, free serine; *M*, free methionine; *A*, A band of serine; A_1 and A_2, two A bands of methionine; *B*, B band. Scale, 2 cm.

20 September 1968

References and Notes

1. K. A. Marcker and F. Sanger, *J. Mol. Biol.* **8**, 835 (1964).
2. J. M. Adams and M. R. Capecchi, *Proc. Nat. Acad. Sci. U.S.* **55**, 212 (1966); R. E. Webster, D. L. Engelhardt, N. D. Zinder, *ibid.*, p. 155; B. F. C. Clark and K. A. Marcker, *J. Mol. Biol.* **17**, 394 (1966); D. Kolakofsky and T. Nakamoto, *Proc. Nat. Acad. Sci. U.S.* **56**, 1786 (1966); J. Lucas-Lenard and F. Lipmann, *ibid.* **57**, 1050 (1967); K. A. Marcker, B. F. C. Clark, J. S. Anderson, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 279 (1967); J. H. Schwartz, *J. Mol. Biol.* **30**, 309 (1967); M. S. Bretscher, *Nature* **218**, 675 (1968); D. Hauschild-Rogat, *Mol. Gen. Genet.* **102**, 95 (1968).
3. G. Zubay, *J. Mol. Biol.* **4**, 347 (1962).
4. D. L. Bergquist and J. M. Robertson, *Biochim. Biophys. Acta* **103**, 579 (1965); M. N. Thang, W. Guschlbauer, H. G. Zachau, M. Grunberg-Manago, *J. Mol. Biol.* **26**, 403 (1967); R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, H. Marquisee, S. H. Merrill, J. R. Penswick, A. Zamir, *Science* **147**, 1462 (1965).
5. R. Monier, M. L. Stephenson, P. C. Zamecnik, *Biochim Biophys. Acta* **43**, 1 (1960).
6. M. W. Nirenberg and J. H. Matthaei, *Proc. Nat. Acad. Sci. U.S.* **47**, 1588 (1961).
7. J. Jarabak, J. A. Adams, H. G. Williams-Ashman, P. Talalay, *J. Biol. Chem.* **237**, 345 (1962).
8. O. Lowry, N. Rosebrough, A. Farr, R. Randall, *ibid.* **193**, 265 (1951).
9. J. J. Kolb and G. Toennies, *ibid.* **144**, 193 (1942).
10. J. C. Sheehan and D. H. Yang, *J. Amer. Chem. Soc.* **80**, 1154 (1958).
11. M. S. Bretscher, *J. Mol. Biol.* **7**, 446 (1963).
12. Machery-Nagel, Düren, Germany.
13. Technical Brochure 66TR 1, Schwarz Bio-Research Inc., Orangeburg, N.Y.
14. K. A. Marcker, *J. Mol. Biol.* **14**, 63 (1965).
15. I thank Miss G. Klein for assistance in the laboratory; Mr. D. Klein, for baker's yeast; Dr. K. W. Mundry, Dr. H. G. Zachau, Dr. S. Ochoa, Dr. M. Grunberg-Manago for their advice and encouragement for my research; and Dr. G. Melchers for his support.