histidine and the scarcity of isoleucine in hemoglobin.

The ratio of relative abundance of tRNA in reticulocytes compared to that in liver is approximately 2 for valine, alanine, glycine, and phenylalanine. It is more difficult, however, to evaluate the translational needs of reticulocytes compared to the liver with respect to these amino acids than it is in the cases of isoleucine and histidine.

There are hazards in a study of tRNA content. The reliability of an assessment of tRNA content is dependent on the quantitative or at least representative extraction of all tRNA species from the cells being studied. It is also dependent on the intactness of all of the tRNA with respect to amino acid acceptance, at least to the extent that the molecules are intact within the cell. That these criteria may not have been fully met in this study is suggested by the sum of acceptance of all amino acids per absorbancy unit of tRNA. The value for tRNA from both sources is substantially less than stoichiometric (1400 to 1800 pmole). This could indicate alteration of some of the tRNA during extraction so that it cannot accept amino acids. Acceptance of amino acids even by some purified individual tRNA species falls far short of 100 percent for reasons that are not fully understood (*8*). It is likely that the tRNA preparations in our study contain fragments of larger nucleic acid molecules, causing a decrease in apparent total acceptance activity but not altering the validity of the results for comparative purposes.

Gilbert and Anderson (*9*) have published determinations of amino acid acceptance by reticulocyte and liver tRNA, though not from the point of view of specialization of tRNA content for hemoglobin synthesis. Their data show some differences from ours, possibly because their preparations were made from supernatants of homogenates centrifuged at 105,000*g* which would not include the tRNA associated with the ribosomes and that shown to be attached to reticulocyte membranes (*10*). In the cases of isoleucine and histidine their results and ours are in close agreement.

Our results are offered as evidence for the specialization of the content of certain tRNA species in reticulocytes for hemoglobin synthesis. Studies in other laboratories have shown a tRNA content in other specialized cells which is consistent with the synthesis of proteins of unusual amino acid composition. These studies include the silk glands of silkworms, which synthesize fibroin that is composed almost entirely of four amino acids (*11*); fibroblasts of healing wounds that make collagen, which is rich in proline (*12*); and the livers of roosters treated with estrogenic hormones causing them to synthesize large amounts of a serine-rich phosphoprotein (*13*). We suggest that in general the relative tRNA content of different kinds of cells in the same animal is similar, but that modification of the content can occur in a manner consistent with unusual amino acid requirements in protein translation. The modifications in some cases are the result of temporary functional adaptations and in other cases (such as reticulocytes) are an aspect of differentiation.

DAVID W. E. SMITH
ANNE L. MCNAMARA
Department of Pathology, Northwestern University Medical School, Chicago, Illinois 60611

References and Notes

1. H. Rahaminoff and H. R. V. Arnstein, *Biochem. J.* **115**, 113 (1969).
2. M. O. Dayhoff, *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Spring, Md., 1969), vol. 4, D-37 to D-65.
3. G. R. Tristram, in *The Proteins*, H. Neurath and K. Bailey, Eds. (Academic Press, New York, 1953), vol. 1A, pp. 181–283.
4. M. Rabinovitz, M. L. Freedman, J. M. Fisher, C. R. Maxwell, *Cold Spring Harbor Symp. Quant. Biol.* **34**, 567 (1969).
5. H. A. Itano, in *Abnormal Hemoglobins in Africa*, J. H. P. Jonxis, Ed. (Blackwell, Oxford, 1965), pp. 3–16.
6. A reticulocytosis of about 80 percent was induced in rabbits by five successive daily injections of neutralized phenylhydrazine solution (6.25 mg per kilogram of body weight per day). The rabbits were bled on day 8, the blood was treated with heparin, and the cells were washed three times with a physiological saline solution. Cells for tRNA were lysed with distilled water, and the lysate was extracted three times in an equal volume of liquified phenol. The aqueous phases of the extractions were combined, and the tRNA was stripped of esterified amino acids by raising the *p*H to 9.5 at 37°C for 30 minutes. After neutralization, the extract was placed on a diethylaminoethyl-cellulose column and eluted successively with 0.02, 0.20, and 2.00*M* LiCl [R. W. Holley, J. Apgar, B. P. Doctor, J. Farrow, M. A. Marini, S. H. Merrill, *J. Biol. Chem.* **236**, 200 (1961)]. The material eluted with 2.00*M* LiCl was precipitated overnight with three volumes of cold ethanol, and the precipitate was collected by centrifugation or on a Gelman GA-8 solvent-resistant membrane filter. The precipitate was dissolved or eluted from the filter in water, and the solution was frozen for storage. For preparation of aminoacyl-tRNA synthetases, reticulocytes were lysed with 0.0025*M* $MgCl_2$ and 0.02*M* tris buffer, *p*H 7.5. The lysate was centrifuged first at 45,000*g* for 30 minutes and then at 105,000*g* for 90 minutes, and the enzymes were precipitated from the resulting supernatant by bringing it to 70 percent saturation with $(NH_4)_2SO_4$. The precipitate, which was washed once with 70 percent $(NH_4)_2SO_4$, was dissolved in a solution of 0.02*M* tris, *p*H 7.5, 0.025*M* KCl, 0.004*M* $MgCl_2$, and 20 percent glycerol. The preparations were stored at —20°C and were prepared frequently. Liver was homogenized in the lysing solutions described, and afterward the above procedures were carried out for the preparation of tRNA and enzymes. The rabbits from which the livers were taken had not been treated with phenylhydrazine. The ratio of absorbancy at 260 to 280 nm of the tRNA preparations was 1.80 to 1.85. The tRNA from both sources eluted as a single peak from a Sephadex G-100 column 100 cm in length.
7. R. Jackson and T. Hunter, *Nature* **227**, 672 (1970); D. Housman, M. Jacobs-Lorena, U. L. Rajbhandary, H. F. Lodish, *ibid.*, p. 913; D. A. Shafritz and W. F. Anderson, *ibid.*, p. 918.
8. J. Apgar, R. W. Holley, S. H. Merrill, *J. Biol. Chem.* **237**, 796 (1962); T. H. Kuo and E. B. Keller, *Fed. Proc.* **27**, 341 (1968).
9. J. M. Gilbert and W. F. Anderson, *J. Biol. Chem.* **245**, 2342 (1970).
10. E. R. Burka, *J. Clin. Invest.* **48**, 1266 (1969).
11. J. P. Garel, P. Mandel, G. Chavancy, J. Daillie, *FEBS Lett.* **7**, 327 (1970).
12. K. W. Lanks and I. B. Weinstein, *Biochem. Biophys. Res. Commun.* **40**, 710 (1970).
13. P. H. Maenpaa and M. R. Bernfield, *Biochemistry* **8**, 4926 (1969).
14. D. W. E. Smith, *J. Biol. Chem.* **241**, 4487 (1966).
15. Supported in part by NSF grant GB 18869. We acknowledge technical assistance by V. Meltzer and W. Gavin.

3 September 1970; revised 19 October 1970 ■

Specific Enzymic Methylation of an Arginine in the Experimental Allergic Encephalomyelitis Protein from Human Myelin

Abstract. *A cytoplasmic enzyme from guinea pig brain was shown to transfer methyl groups from* S*-adenosylmethionine to only one of 19 arginine residues in the basic protein from human brain. The products were* ω-N*-monomethylarginine and* ω-N,N′*-dimethylarginine. These methylated arginines are adjacent to the main encephalitogenic determinant in the protein. Methylation may aid in the transfer of this region of the protein into the nonpolar environment within myelin and in maintaining the integrity of myelin.*

Myelin from the central nervous system contains an unusual basic protein that will induce experimental autoimmune encephalomyelitis. From their studies on the plaques of multiple sclerosis Adams and Hallpike (*1*) consider it to be the most vulnerable part of myelin. During sequence studies (*2*) on the encephalitogenic basic protein from human brain microheterogeneity was observed at position 107. An arginine at this position occurs either in the dimethylated, monomethylated, or unmethylated form (*3*). Eylar (*4*)

has reported a modified arginine at residue 107 in the bovine protein.

Although methylated arginine had not hitherto been identified in an amino acid sequence, the enzymic methylation of arginine in proteins has been extensively studied (*5*, *5a*). A cytoplasmic enzyme from calf thymus transferred methyl groups from *S*-adenosylmethionine to arginine in histones. Liver, but surprisingly not thymus, histones contain methylated arginine (*6*). Paik and Kim (*7*) found in a survey of a number of tissues that brain had the highest level of this arginine methylase (*8*), but that in brain an unidentified protein other than histone was acting as the receptor for methyl transfer. We now present evidence for the specific methylation of an arginine residue in the encephalitogenic basic protein from human brain.

Crude arginine methylase was prepared by centrifugation of a homogenate of guinea pig brain at 105,000*g* for 60 minutes followed by precipitation of the enzyme from the supernatant with 30 percent $(NH_4)_2SO_4$ as described by Paik and Kim (*5*). The encephalitogenic protein (*2*) from human brain (3 mg) was incubated with 0.20 mg of enzyme and 0.30 μc of *S*-adenosyl-L-[methyl-^{14}C]methionine (International Chemical and Nuclear; specific activity, 55 μc/μmole), in a total volume of 0.5 ml of 0.1*M* sodium phosphate buffer, *p*H 6.6. After incubation for 2 hours the protein was precipitated and washed as described (*5*). The precipitate contained 8970 dpm (3.18 pmole of [^{14}C]methyl per minute per milligram of enzyme). The precipitate was hydrolyzed with constant boiling HCl, and the hydrolyzate was fractionated on a Technicon C-2 resin; of the total radioactivity 83 percent was recovered in two zones which corresponded to monomethylarginine (MMA) and dimethylarginine (DMA) (*3*). The ratio of [^{14}C]methyl in these two zones was respectively 2.86:1. In contrast in the batch of protein (*3*) used as the receptor the ratio of arginine to MMA to DMA was 1:6:10. Thus it appeared that the enzyme preferentially transferred methyl groups to arginine rather than to the monomethyl form although the latter was present in the receptor in a sixfold greater amount.

Other samples incubated under identical conditions except with twice as much enzyme were digested with trypsin or pepsin, and the products were fractionated by electrophoresis and chromatography (*9*). The peptide maps were placed in contact with x-ray film for 16 days and then stained with ninhydrin. A typical autoradiogram of a tryptic digest of the labeled protein is shown in Fig. 1 with the positions of the tryptic peptides outlined. Only one peptide, T23, was labeled; no trace of radioactivity was found elsewhere on the map. Trypsin does not cleave bonds involving methylarginine (*3*); if therefore any arginine other than 107 had been labeled, an additional radioactive spot, not found in the position of a peptide on a normal map, would have been present. The sequence of peptide T23 is shown in Fig. 2. It arises from the fission of a Lys-Gly (*10*) bond at residues 105–106 and an Arg-Phe bond at residues 113–114. To further substantiate the localization of the label on Arg-107, the radioactive peptide was cut out, before staining with ninhydrin, and was digested with thermolysin (*9*). The label was associated with a peptide having mobility, at *p*H 2.1, relative to lysine of 0.91 and having an R_F relative to leucine of approximately 0.39. In that Gly-MMA-Gly and Gly-DMA-Gly have these chromatographic and electrophoretic properties (*3*), they would not have been separated from each other.

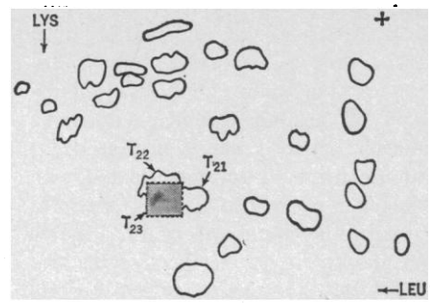

Fig. 1. Autoradiogram of a peptide map of a tryptic digest of the encephalitogenic basic protein from human brain after ^{14}C-methylation by arginine methylase. Electrophoresis was at *p*H 6.5 in pyridine acetate; the position of a lysine (Lys) marker is shown. Electrophoresis was followed by chromatography in a system consisting of *n*-butanol, pyridine, acetic acid, and water (15:10:3:12, by volume); the position of a leucine (Leu) marker is shown. The peptides were stained with ninhydrin and traced onto the autoradiogram. Only one spot of radioactivity was found in the position of peptide T23. The area in the square was cut out and digested with thermolysin.

$$\underset{105}{\text{Lys-Gly-}}\left\{\begin{matrix}\text{DMA}\\\text{MMA}\\\text{Arg}\end{matrix}\right\}\text{-Gly-Leu-Ser-Leu-}\underset{110}{\text{Ser-}}$$

$$\underset{115}{\text{Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-}}\underset{120}{\text{Arg}}$$

Fig. 2. Amino acid squence of residues 105 to 122 in the encephalitogenic basic protein from human brain. DMA is ω-*N*,*N*′-dimethylarginine and MMA is ω-*N*-monomethylarginine. Tryptic peptide T23 resulted from fission of a Lys-Gly bond at 105–106 and an Arg-Phe bond at 113–114. The encephalitogenic determinant is underlined.

Additional evidence for the localization of the [^{14}C]methyl group on Arg-107 came from an autoradiogram of a peptide map of the peptic digest. The strongest radioactive spot was associated with a peptide with the sequence Pro-Arg-Thr-Pro-Pro-Pro-Ser-Gln-Gly-Lys-Gly-(MMA or DMA or Arg)-Gly-Leu-Ser-Leu (*2*). After acid hydrolysis of the peptide, the radioactivity was found mainly associated with the MMA zone with only a little in the DMA zone. Some undigested and partially digested protein was found on the electrophoresis strip.

Thus we have demonstrated that the encephalitogenic basic protein was specifically methylated at only one of 19 arginines in the protein. Since the protein in aqueous solution appears to lack secondary structure (*11*), we conclude that arginine methylase probably recognizes some feature of the amino acid sequence in the proximity of Arg-107. The recognition site could be quite small; in the transfer of *N*-acetylglucosamine residues to a protein the transferase appears to recognize the short sequence Asn-X-Ser (or Thr) (*12*). Studies with synthetic polypeptides should enable the delineation of the required sequence in the receptor for arginine methylase. Some histones and cytochromes have been shown to contain methylated lysines at one or two specific residues (*13*). However, there is at present no obvious homology between the sequences around these lysines in the two types of proteins. Paik and Kim have shown that a lysine methylase is located in nuclei (*14*).

The methylated arginine occurs close to the main encephalitogenic determinant in the protein from human brain (Fig. 2). This determinant was isolated and characterized (*9*), and its structure has been confirmed by chemical synthesis of a peptide encephalitogen (*15*). There is a striking structural resemblance between the encephalitogenic determinant (*2*) and the requirements for a binding site for 5-hydroxy-

tryptamine (*16*) in a neuronal membrane. It was postulated (*2*) that the clinical symptoms of hind quarter paralysis and incontinence typical of experimental allergic encephalomyelitis could be due to an immunopharmacological block of a receptor site for 5-hydroxytryptamine in certain regions of the central nervous system that have a high concentration of 5-hydroxytryptamine nerve terminals.

The basic protein is closely associated with lipids in myelin and is located in the intraperiod line in the lamellar structure of myelin (*17*). One of several possible roles for the arginine methylase could be to aid in the transfer of this region of the basic protein into the nonpolar environment within myelin, because the attachment of methyl groups would raise arginine on the hydrophobicity scale of Tanford (*18*).

Vitamin B_{12} deficiency in man can cause "subacute combined degeneration" in the central nervous system. This disease is characterized by the presence of malformed ("spongy") myelin, and it has always been difficult to rationalize the role of vitamin B_{12} in preventing this disease (*19*). However, because B_{12} takes part in the metabolism of *S*-adenosylmethionine (*20*), which is the source of methyl groups for the methylation of arginine in the basic protein of myelin, it is possible that incomplete methylation could result in malformed myelin. We predict that the basic protein from "spongy" myelin in subacute combined degeneration will be found to be deficient in dimethylarginine.

The coexistence in brain of the unmethylated and partially methylated basic protein with a cytoplasmic arginine methylase raises important questions concerning the control and site of methylation. Are the three forms of the protein located in different regions of the oligodendroglial neuron complex, or does the partially methylated form represent an intermediate stage in the formation of myelin? We suggest that arginine methylase and the encephalitogenic basic protein may have an important functional role in maintaining the myelin sheath in the central nervous system.

G. S. BALDWIN
P. R. CARNEGIE
Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, Victoria 3052, Australia

References and Notes

1. C. W. M. Adams and J. F. Hallpike, *Guy's Hosp. Rep.* **118**, 263 (1969).
2. P. R. Carnegie, *Nature*, in press.
3. G. S. Baldwin and P. R. Carnegie, in preparation. The methylated arginines were isolated and subjected to alkaline hydrolysis. Quantitative ion-exchange chromatography and paper electrophoresis and chromatography established the products as monomethylamine and ornithine. ω-*N*,*N*′-Dimethylarginine and ω-*N*-monomethylarginine had elution volumes relative to arginine of 0.92 and 0.97, respectively, on a Technicon C-2 resin.
4. E. H. Eylar, *Proc. Nat. Acad. Sci. U.S.* **67**, 1425 (1970).
5. W. K. Paik and S. Kim, *J. Biol. Chem.* **243**, 2108 (1968); *Arch. Biochem. Biophys.* **134**, 632 (1969); *J. Biol. Chem.* **245**, 88 (1970).
5a. M. Friedman, K. H. Shull, E. Farber, *Biochem. Biophys. Res. Commun.* **34**, 857 (1969); A. M. Kaye and D. Sheratzky, *Biochim. Biophys. Acta* **190**, 527 (1969).
6. W. K. Paik and S. Kim, *Biochem. Biophys. Res. Commun.* **40**, 224 (1970).
7. ———, *J. Neurochem.* **16**, 1257 (1969).
8. Paik and Kim have referred to this enzyme as "Protein methylase I" (*5*). We prefer the name "arginine methylase" to avoid confusion with other enzymes known to methylate lysine (*13*), histidine [E. L. Gershev, G. W. Hapett, G. Vidali, V. G. Allfrey, *J. Biol. Chem.* **244**, 4831 (1969)], and carboxyl groups [W. K. Paik and S. Kim, *ibid.* **245**, 1806 (1970)] in proteins.
9. P. R. Carnegie, *Nature* **223**, 958 (1969); V. A. Lennon, A. V. Wilks, P. R. Carnegie, *J. Immunol.* **105**, 1223 (1970).
10. Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Gly, glycine; Glu, glutamic acid; Gln, glutamine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan.
11. F. B. Palmer and R. M. C. Dawson, *Biochem. J.* **111**, 629 (1969); E. H. Eylar and M. Thompson, *Arch. Biochem. Biophys.* **129**, 468 (1969); L.-P. Chao and E. R. Einstein, *J. Neurochem.* **17**, 1121 (1970); Y. Oshiro and E. H. Eylar, *Arch. Biochem. Biophys.* **138**, 606 (1970).
12. R. L. Jackson and C. H. W. Hirs, *J. Biol. Chem.* **245**, 624 (1970).
13. R. J. De Lange, D. M. Fambrough, E. L. Smith, J. Bonner, *ibid.* **244**, 5669 (1969); R. J. De Lange, A. N. Glazer, E. L. Smith, *ibid.*, p. 1385; *ibid.* **245**, 3325 (1970); Y. Ogawa, G. Quagliarotti, J. Jordan, C. W. Taylor, W. C. Starbuck, H. Busch, *ibid.* **244**, 4387 (1969).
14. W. K. Paik and S. Kim, *Biochem. Biophys. Res. Commun.* **29**, 14 (1967).
15. F. C. Westall, A. B. Robinson, J. Caccam, J. Jackson, E. H. Eylar, *Nature* **229**, 22 (1971).
16. J. R. Smythies, F. Benington, R. D. Morin, *Neurosci. Res. Progr. Bull.* **8**, 117 (1970).
17. J. P. Dickinson, K. M. Jones, S. R. Aparicio, C. E. Lumsden, *Nature* **227**, 1133 (1970).
18. C. Tanford, *J. Amer. Chem. Soc.* **84**, 4240 (1962).
19. J. Bebin, in *Pathology of the Nervous System*, J. Minckler, Ed. (McGraw-Hill, New York, 1968), vol. 1, pp. 1044–48.
20. H. A. Barker, in *The Vitamins*, W. H. Sebrell and R. S. Harris, Eds. (Academic Press, New York, 1968), vol. 2, pp. 184–212.
21. We thank G. G. Luxford for technical assistance and the National Multiple Sclerosis Society, Australian Research Grants Committee, and the Multiple Sclerosis Society of Victoria for financial support. We thank Drs. R. F. Kibler, S. Shapira, and E. H. Eylar for comments on the sequence of the human protein.

27 October 1970; revised 7 December 1970 ■

Induction of Microsomal Oxidase in F_1 Hybrids of a High and a Low Oxidase Housefly Strain

Abstract. *Microsomal oxidase activity is increased up to sixfold in dieldrin-resistant houseflies exposed to sublethal doses of dieldrin. The net increase in enzyme activity is greater in a strain initially high in oxidase activity than in a strain low in this respect, and this tendency is carried into the F_1 hybrids of the strains.*

The phenomenon of insect resistance to insecticides is generally attributed in considerable part to increased detoxication of the toxic agent. Early evidence of this was provided by Perry and Hoskins (*1*) who demonstrated that DDT-resistant houseflies converted DDT to less toxic DDE. Others have shown that DDT-resistant houseflies contain more of the enzyme DDT-dehydrochlorinase than susceptible strains (*2*) and that microsomes isolated from resistant houseflies are more active in hydroxylating naphthalene (*3*). Numerous reports have confirmed and extended this concept.

The microsomal oxidases of the housefly are important in resistance to several types of insecticides (*4*), and it is now known that this system becomes more active in insects exposed to enzyme inducers such as DDT (*5*) and dieldrin (*6*, *7*). In our study of induction by dieldrin in the housefly (*7*) we observed a greater net response in the microsomal oxidase activity of a high oxidase strain than in a low oxidase strain. This indicated that high oxidase strains have a greater tendency to be induced and suggested that a test of this assumption would be to cross a high and a low oxidase strain and measure induction in the hybrids. We now report that such hybrids are induced by dieldrin to produce microsomal oxidases of intermediate activity relative to the parents. We interpret these results to mean that the high oxidase strain differs from the low oxidase strain in the regulation of enzyme level, possibly by possessing more genes or gene sequences for increased production of detoxifying enzymes.

The dose of dieldrin necessary for in-