Reports

Is Memory a Matter of Enzyme Induction?

Abstract. Variations in cholinesterase and RNA concentrations and in levels of neural activity have been linked to learning. These three groups of experimental evidence suggest that the basis of memory lies in an increase of the concentrations of enzymes associated with transmitter substances, as a long-lasting effect of stimulation. Biological precedent exists in microbial physiology.

Enzyme induction has been defined as "the increase in the ratio of the rate of synthesis of a given enzyme to the rate of synthesis of total cell protein resulting from exposure of cells to compounds (inducers) which are identical or structurally related to the substrates of the given enzyme" (*1*). If one assumes that induction, first observed in certain microbial cells, occurs in neurons during the course of functional activity of nervous systems, memory may be defined on a molecular level and in terms subject to test.

Stimulation of a neuron causes the release of a transmitter substance. If induction occurs in neurons, the inducer would therefore be the transmitter substance, and the enzyme induced would be the enzyme associated with the synthesis or inactivation of the transmitter. By analogy with microbial induction, neuronal transmitter enzyme induction may affect the concentration of an entire enzyme system, including enzymes which synthesize, inactivate and bind the transmitter in a storage compound. Such an induction hypothesis fits three kinds of experimental evidence.

The first group of data relates the concentration of acetylcholinesterase to some parameters of behavior, including total quantity of stimulation (*2*) and genetic differences in ability to learn a maze (*3*). The enzyme participates in a reaction sequence outlined by Nachmansohn (*4*), beginning with the synthesis of the transmitter acetylcholine, followed by its binding with a compound as yet unidentified, its release by stimulation, its action on a receptor, and its hydrolysis by the esterase. An enzyme induction hypothesis would lead one to expect that the esterase and other components of the system would increase in concentration with increases in the total quantity of stimulation. It would follow that each nerve impulse would release from the bound form more acetylcholine than an equivalent impulse released before induction. Genetic ability to learn, demonstrated as a variable linked to enzyme concentrations (*3*), would be expected to act through induction to make animals with initially higher concentrations learn in fewer trials than animals with initially lower concentrations of enzymes of the transmitter systems.

The second kind of evidence is centered on increases in RNA in "active" cells (*5*, *6*). It would be expected, if enzyme induction occurred, that an increase in momentarily free substrate, whether exogenous as in microbial cells or endogenous as in neurons, would not only increase the enzymes associated with metabolism of the substrate but would also cause a rise in the concentration of RNA. Hyden has reported such increases (*5*) and in still more elegant analyses subsequently found changes in ratios of specific nucleotides in RNA after learning (*7*). The interpretation he has put upon the early results is that specific frequencies of nerve impulses may modify protein structure by modifying nucleotide sequence in RNA. Such an interpretation presents formidable difficulties for theory, because it postulates an orderly and nonrandom recoding of genetic material.

Recent developments in genetics and immunology support the point of view that only by such random processes as radiation and transduction are nucleic acids modified. Immunology in particular has dealt with the "instructive" intervention of antigens in protein synthesis, only to have theory forced by experiment back to the position that nucleic acids are not changed (*8*). As antigen is part of the experience of a cell, modifying its protein production at some level superficial to the genetic level, it seems profitable to consider nerve impulses the experience of neurons which can modify their protein production at a level superficial to RNA nucleotide sequence.

The third kind of evidence is that concerning variations in rate of learning with variations in absolute number of impulses. When the number is presumably reduced by interrupting post-trial consolidation with electroconvulsive shock, rate of learning declines (*9*). When the level of activity is raised by such drugs as strychnine (*10*) or lowered by barbiturates (*11*), rate of learning rises in the first case and falls in the second. Induction, by its dependence on total quantity of stimulation as a measure of quantity of endogenous substrate liberated, would account for these data.

The hypothesis of induction implies a response to repeated stimulation at myoneural junctions as well as in central synapses. If such were the case, and it is plausible to test, the isolated tail of the planarian would be expected to retain a peripherally conditioned response without relying on tail RNA for relocation of its memory in a regenerated head (*12*).

Among the difficulties of an induction hypothesis is the demonstrated existence of nonlearning neurons which participate in unconditioned reflexes. Why are they incapable of modification beyond the brief span of posttetanic potentiation? Another difficulty, which may lead to analyzing the first, is the sequence of events setting an upper limit on the concentration of transmitter enzyme systems. Thus recent studies of the mechanism underlying induction phenomena imply processes operating by the release or repression of parts of the genetic material (*13*). In any case, the biological generality of induction suggested by such a level of response improves the plausibility of the hypothesis that induction is responsible for memory in neural tissue. From one primal response different specialized cells may achieve many differing arguments for survival. For neurons in particular, induction could provide high information capacity without the entropic expense of recoding protein, because the sheer number of cells would work with the range of enzyme concentrations to define an information capacity as rich as the variety of behavior (*14*).

C. E. SMITH

San Jose State College, San Jose, California

References and Notes

1. M. Cohn, J. Monod, M. R. Pollock, S. Spiegelman, R. Y. Stanier, *Nature* **172**, 1096 (1953).
2. R. Liberman, *Science* **135**, 372 (1962).
3. D. Krech, M. R. Rosenzweig, E. L. Bennett, *J. Comp. Physiol. Psychol.* **53**, 509 (1960).
4. D. Nachmansohn, *Science* **134**, 1962 (1961).
5. H. Hyden and A. Pigon, *J. Neurochem.* **6**, 57 (1960).
6. J. A. Deutsch, *Ann. Rev. Physiol.* **24**, 270 (1962).
7. H. Hyden and E. Egyhazi, *Proc. Natl. Acad. Sci. U.S.* **48**, 1366 (1962).
8. F. M. Burnet, *Perspectives Biol. Med.* **3**, 447 (1960).
9. C. W. Thomson, J. C. McGaugh, C. E. Smith, W. J. Hudspeth, W. H. Westbrook, *Can. J. Psychol.* **15**, 67 (1961).
10. J. L. McGaugh and L. Petrinovitch, *Am. J. Psychol.* **72**, 99 (1959).
11. M. Moroz, *J. Comp. Physiol. Psychol.* **52**, 172 (1959).
12. W. C. Corning and E. R. John, *Science* **134**, 1363 (1961).
13. S. M. Beiser and S. W. Tanenbaum, *Nature* **189**, 659 (1961).

14. I am indebted for critical analysis and useful suggestions to several colleagues, particularly Drs. Harold J. DeBey and Calvin W. Thomson.

27 July 1962

Fatty Acids in Pollen of Some Coniferous Species

Abstract. Fatty acids in pollen of five coniferous species were isolated and analyzed by gas-liquid chromatography. It was found that 0.76 to 0.89 percent of the dry weight of pollen was fatty acid in three species of *Pseudotsuga* and 1.25 to 1.33 percent in two species of *Pinus*. Major components in *Pseudotsuga* were oleic, palmitic, and linoleic acids, whereas in *Pinus* they were linolenic, oleic, palmitic, and stearic acids.

In the course of developing methods for preserving pollen for plant hybridization, the chemical composition of pollen from various conifers was determined. Fatty substances in pollen have received little attention because of the small amounts available for analyses (*1*). This paper, for the first time, reports the quantitative and qualitative determinations of fatty acids in three species of *Pseudotsuga* and two species of *Pinus*. There may be a correlation between the chemical findings and the phylogenetic relationships of the two genera studied.

Pollen of Formosan Douglas fir, *Pseudotsuga wilsoniana* Hay, was collected in Ta Chia Chi, Taiwan, in the middle of February, 1962. Branches of big-cone Douglas fir, *Pseudotsuga macrocarpa* (Vasey) Mayr, bearing mature male flowers, were shipped from southern California to Corvallis in the early part of April, in cartons which contained moist paper, and the pollen sac was forced to dehisce in the laboratory. Mature pollen of Douglas fir, *Pseudotsuga menziesii* (Mirb) Franco, was gathered in Corvallis from a single tree by a vacuum pollen collector in early April. Pollen of ponderosa pine, *Pinus ponderosa* Dougl., and lodgepole pine, *Pinus contorta* Dougl., was obtained from trees on the Oregon State University campus in the early part of May. That all pollen samples were highly viable at the time of extraction was indicated by germination tests.

Duplicate samples of 1 gram of fresh pollen were inactivated in 10 ml of boiling isopropanol; after cooling, 20 ml of peroxide-free diethyl ether was added. Extraction was conducted at room temperature (21°C), for 16 hours with occasional stirring, followed by two successive extractions of ether for 4 hours. Extracts were filtered with the aid of vacuum, combined, and washed; the solvent was removed in a rotary vacuum evaporator. The extracted fatty substances were saponified by 2 percent ethanolic sodium hydroxide for 2 hours; the ethanol was removed by a stream of nitrogen gas. The soap was dissolved in water, and the nonsaponifiable material was removed by hexane. The soap solution was acidified, and the fatty acids were recovered in ether and methylated with diazomethane.

The mixtures of the methyl esters of the fatty acids were separated, in a temperature-programmed gas chromatograph by two columns which contained 10 percent and 20 percent diethyleneglycol succinate on acid-washed Chromosorb w (90 to 100 mesh). The identification of components was conducted by cochromatographic technique with pure methyl esters or by matching retention time of known mixtures. The quantitative analysis of the components was obtained by weighing the material from the peaks after each column was calibrated against the standard mixture.

The total fatty acid content, expressed as percentage of dry weight, of the pollen of each species was as follows: Douglas fir, 0.79; Formosan Douglas fir, 0.89; big-cone Douglas fir, 0.76; ponderosa pine, 1.33; and lodgepole pine, 1.25. The quantitative analysis of fatty acid in each species is shown in Table 1. Oleic, palmitic and linoleic acids are the major components in the Douglas firs, and linolenic, oleic, palmitic, and stearic acids are the major components in the pines.

It is interesting to note the similarity between the fatty acid composition of Douglas fir and Formosan Douglas fir and between the two pines, while the composition of big-cone Douglas fir stands as an intermediate between the other two species of Douglas fir and pines.

The unknown component in big-cone Douglas fir pollen is not margaric acid, a saturated C_{17} fatty acid, since the unknown formed a shoulder when margaric acid was cochromatographed. The unknown could be an unsaturated C_{16} fatty acid, for the peak disappeared when the mixture was hydrogenated and an increase of palmitic acid was observed.

Taxonomic relationships of plant waxes which were analyzed by gas chromatography were shown by Eglinton (*2*), and characterization of plant families by major fatty acids has been indicated in the literature (*3*, *4*). Ivanov considers oil content to be an inherited characteristic of plants whereas iodine value or composition of unsaturated fatty acids changes with climate (*3*). The data presented are

Table 1. Distribution of fatty acids (as percentage, by weight, of methyl esters) in the pollen of five species of Pinaceae.

Fatty acid	Douglas fir	Formosan Douglas fir	Big-cone Douglas fir	Ponderosa pine	Lodgepole pine
Caproic, C_6			0.2		
Caprylic, C_8			0.3	0.5	0.8
Capric, C_{10}			0.6	2.5	1.8
Lauric, C_{12}			0.5	4.9	6.1
Myristic, C_{14}	0.2	0.1	0.8	2.0	1.8
Palmitic, C_{16}	20.9	26.5	26.4	17.6	13.4
Palmitoleic, C_{16}*	0.2	0.2	0.2		
Unknown			1.7		
Stearic, C_{18}	2.7	2.5	15.6	10.9	12.2
Oleic, C_{18}*	62.2	52.9	39.0	23.1	16.5
Linoleic, C_{18}†	11.9	16.4	8.0	5.4	4.4
Arachidic, C_{20}	0.2				
Linolenic, C_{18}‡	0.9	0.9	4.5	24.1	31.5
Eicosenoic, C_{20}*	0.4	0.3	1.3	2.5	2.9
Behenic, C_{22}	0.3	0.2	0.9	3.1	3.1
Erucic, C_{22}*				3.6	3.5

* One carbon-to-carbon double bond. †Two carbon-to-carbon double bonds. ‡Three carbon-to-carbon double bonds.