A paper entitled "Pharmakologie des Tetrahydropapaverolins und seine Entstehung aus Dopamin" will appear in *Arch. exp. Path. Pharmak.*, 248, 387 (1964).

Tetrahydropapaveroline was a gift from the Wellcome Laboratories, London.

P. Holtz
K. Stock
E. Westermann

Pharmacological Institute,
University of Frankfurt,
Frankfurt-am-Main,
Germany.

[1] Holtz, P., and Heise, R., *Arch. exp. Path. Pharmak.*, **191**, 87 (1938).
[2] Holtz, P., Stock, K., and Westermann, E., *Arch. exp. Path. Pharmak.*, **246**, 133 (1963).
[3] Holtz, P., and Westermann, E., *Arch. exp. Path. Pharmak.*, **231**, 311 (1957).
[4] Pyman, F. L., *J. Chem. Soc.*, **95**, 1610 (1909).
[5] Laidlaw, P. P., *J. Physiol.* (Lond.), **40**, 480 (1910).
[6] Stahl, E., *Dünnschicht Chromatographie* (Springer-Verlag, Heidelberg, 1962).
[7] Black, J. W., and Stephenson, S., *Lancet*, ii, 311 (1962).
[8] Holtz, P., and Credner, K., *Arch. exp. Path. Pharmak.*, **200**, 356 (1942).
[9] Goldenberg, M., Faber, M., Alston, E. J., and Chargaff, E. C., *Science*, **109**, 534 (1949).
[10] Burn, J. H., and Rand, M. J., *Brit. J. Pharmacol.*, **13**, 471 (1958).

Function of *N*-Acetyl Aspartic Acid in the Brain : Effects of Certain Drugs

N-Acetyl-L-aspartic acid (NAA) was discovered by Tallan *et al.* in 1956 and shown to be unique in its restricted localization to the brain of vertebrate species and in its very high concentration in that organ[1]. Occurring at a level of approximately 1 mg/g of brain, NAA is second only to glutamic acid in the level of free amino-acids in this tissue. Numerous attempts, using a variety of drugs and experimental procedures, to change the concentration of NAA in the brain have met with little success. Thus, electric shock, insulin shock, ammonia intoxication, and the administration to animals of tranquillizers, central nervous system depressants or convulsants, all failed to alter significantly the concentration of NAA in the brain[2–4].

It was reported recently that several drugs, classified as monoamine oxidase (MAO) inhibitors, when administered to rats appeared to produce a rise in the concentration of NAA in the brain[5]. As part of a larger study in this laboratory designed to disclose the function of this unusual amino-acid, it was decided to repeat and extend this observation.

In our investigations, *Ha/ICR* Swiss mice were used. Drugs were administered intraperitoneally as six daily injections, except for hadacidin (*N*-formyl hydroxyaminoacetic acid), which was given as a single dose, and 5-hydroxytryphophan (5HTP) and dihydroxyphenylalanine (DOPA), which were given every 3 h for a total of four injections. The animals were killed by cervical dislocation 4 h after the last injection of drug; the brain tissue was removed and deproteinized. The NAA content of the brain extract was determined essentially by the method of Fleming[6]. This procedure utilizes a kidney deacylase preparation to convert NAA to acetate and aspartate, oxalacetic transaminase to convert aspartate to oxaloacetate, and malic dehydrogenase to convert oxaloacetate to malate. Since the last reaction utilizes NADH, the amount of NAA in the sample can be calculated from the spectrophotometrically determined extent of oxidation of NADH to NAD.

The results of these experiments (Table 1) suggest a relationship between NAA and 5-hydroxytryptamine (5HT), since: (*a*) many agents that produce a rise in the level of 5HT in the brain also produce an increased concentration of NAA: (*b*) although some of the agents are monoamine oxidase inhibitors and cause a rise in catecholamines as well as 5HT in the brain, the administration of DOPA, in contrast to 5HTP, had no effect on the level of NAA; (*c*) both LSD-25 (an antagonist to 5HT in peripheral systems) and reserpine (which lowers the brain-level of 5HT) produce a fall in the amount of NAA in the brain. In addition, it should be noted that no correlation exists between brain-levels of NAA and inhibition of monoamine oxidase, since isoniazid caused a rise in the amino-acid concentration but is not an MAO inhibitor, while tranylcypramine ('Parnate') is an inhibitor of MAO and yet does not significantly change the level of NAA in the brain.

Table 1. Effect of Drugs on the Content of *N*-Acetylaspartic Acid (NAA) in the Brain of the Mouse

Drug	Dose (mg/kg)	NAA content of brain μg/g (mean value ± S.D.)	Probability (calculated from *t*-test)
Saline (5)*	—	974 ± 41	—
Iproniazid (9)	100	1,415 ± 245	0·0125
*JB*516 ('Catron') (5)	12	1,120 ± 77	0·025
Tranylcypramine ('Parnate') (10)	15	1,013 ± 82	Not significant
Isoniazid (5)	110	1,339 ± 147	0·005
Imipramine (10)	25	1,165 ± 138	0·005
L-5HTP (10)	50	1,205 ± 79	0·005
L-DOPA (5)	50	980 ± 106	Not significant
Reserpine (10)	0·75	825 ± 114	0·05
LDS-25 (5)	0·25	776 ± 97	0·015
Hadacidin† (5)	100	796 ± 67	0·005

* Number in brackets refers to number of animals.
The following drugs had no effect: pentobarbital, prochlorperazine ('Compazine'), amphetamine, atropine, 4-hydroxybutyric acid, γ-butyrolactone, β-methyl aspartate and NAA.
† The hadacidin was kindly supplied by Dr. H. Shigeura, of Merck, Sharp and Dohme.

The inability of administered NAA to affect the endogenous level of NAA in the brain suggests that the amino-acid cannot pass the blood-brain barrier; this conclusion also is supported by a subsequent experiment in which it was found that the intraperitoneal injection of isotopically labelled NAA did not cause an appearance of labelled NAA in the brain.

Experiments are in progress in an attempt to understand the suggested relationship between 5HT and NAA.

This work was supported by a U.S. Public Health Service research grant (*B*-2291) and a training grant (5-*T*1-*GM*-59-04).

J. C. McIntosh*
J. R. Cooper†

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Conn.

* Predoctoral trainee of the Public Health Service.
† Career Development Awardee of the Public Health Service.

[1] Tallan, H. H., Stein, W. H., and Moore, S., *J. Biol. Chem.*, **219**, 257 (1956).
[2] Du Ruisseau, J. P., *Canad. J. Biochem. Physiol.*, **38**, 763 (1960).
[3] Tews, J. K., Cater, S. H., Roa, P. D., and Stone, W. E., *J. Neurochem.*, **10**, 641 (1963).
[4] Okomura, N., Otsuki, S., and Nasu, H., *J. Biochem.* (Tokyo), **46**, 247 (1959).
[5] Mussini, E., and Marcucci, F., in *Amino Acid Pools*, edit. by Holden, J. P., 465 (Elsevier Pub. Co., 1962).
[6] Fleming, M. C. (personal communication).

HÆMATOLOGY

Inherited Variants in Serum Ceruloplasmins of the Pig

By means of chromatography and electrophoresis on starch gel, Morell and Scheinberg[1] showed at least four different molecular components of ceruloplasmin in preparations from the plasma of 9,109 human donors. Then McAlister *et al.*[2] presented evidence for multiple ceruloplasmin components in human sera. Using vertical starch-gel electrophoresis to resolve the ceruloplasmins combined with direct chemical and enzymatic analysis on the gel, they defined five distinct proteins having one or