Meetings

United States–Japan Committee on Scientific Cooperation: Neurochemistry Conference

Under the auspices of the joint United States-Japan Committee on Scientific Cooperation, a conference on the function and metabolism of the nervous system took place 11–15 October 1965, at Ohiso, Japan. The scientific cooperation program between the two countries was initiated in 1961 to design and support studies on subjects of mutual interest. This neurochemistry conference was the third in the field of research on the nervous system under the program and brought together 8 American and 9 Japanese participants and 15 Japanese observers. There was no serious language barrier, because most of the Japanese participants and observers had studied in the United States. All the reports were presented in English.

The first session dealt with lipids in the nervous system. J. Folch-Pi (Belmont, Massachusetts) reviewed the structure, composition, and biochemistry of myelin. He discussed the electron-microscopic evidence of the protein-lipid, double-layer structure of myelin and the chemical components of myelin. The lipid part contains cholesterol, cerebrosides, and phosphatides, and all its proteins are soluble in a mixture of chloroform and methanol. The protein obtained from isolated myelin is not identical in composition with the proteolipids obtained from whole white matter because the individual lipids of the preparation influence the extractability of the protein component. The linkage between protein and phospholipid is of various types, such as Van der Waals, coulombic, or ionic; perhaps the most stable is that between proteins and tri-phosphoinositides, which is a saltlike linkage.

K. Hayashi (Maebashi) discussed the possibility that di- and tri-phosphoinositides play a role in excitation. These compounds show a remarkably high turnover, which is stimulated by sodium ions and inhibited by ouabain. They show a very rapid post-mortem depletion.

T. Yamakawa (Tokyo) classified glycolipids into two groups, ceramide hexosides and mucolipids (gangliosides). The composition of these compounds and the ratios of kerasine, phrenosine, and cerebroside sulfuric ester varied in the brains of various species. These compounds are metabolically active, as glucose or galactose is rapidly incorporated into them.

T. Taketomi (Tokyo) reported on obtaining brain-specific antigens by complexing various lipid haptens with proteins. Artificial antigens can be used to study the immunological activity of lipids, for the immunological study of membrane architecture, and for clarifying the possible role of immunological mechanisms in demyelinating diseases.

As the first speaker on the topic of amino acids and proteins, Y. Tsukada (Tokyo) discussed the relationship between glutamate and ammonia metabolism and brain function. He found that conditioned and unconditioned stimuli increased the concentration of ammonia in the brain, with a return to normal within 60 seconds after the stimulation. No increase occurred when a second stimulus was applied within 2 hours after the first stimulation. Prolonged stimulation resulted in an increase in glutamine in the brain, which can serve for ammonia removal. Enzymes for glutamate metabolism were shown to be localized mainly in mitochondria. Tsukada also described experimental phenylketonuria induced by a high-phenylalanine diet, which resulted in decreased intellectual performance in monkeys. With increasing phenylalanine in the brain, γ-aminobutyric acid was decreased.

A. Ames, III (Boston) summarized his studies of the intracellular and extracellular spaces and the distribution of electrolytes in retina. Incubated, isolated rabbit retina could produce electrical responses to photostimulation, maintain physiological electrolyte distribution, and preserve histological structure for 1 or 2 hours. Glutamate caused swelling, with an increase in sodium, calcium, and cell water.

M. Kohsaka (Okayama) reported on the composition of a low-phenylalanine diet made of available Japanese foods and used in the treatment of phenylketonuria. Such a diet resulted in improvement in patients even if they were over 3 years old. Kohsaka also described changes in the diet for bringing it slowly back to normal. K. Taniguchi (Osaka) showed evidence for the enhanced metabolism of phenylalanine to *o*-hydroxyphenylacetic acid via phenylpyruvic acid in phenylketonuric patients.

G. Takagaki (Tokyo) discussed the accumulation of D-aspartate in brain slices. This accumulation is accompanied by potassium ions, and asparatate is accumulated in preference to D-glutamate. Aspartate inhibited the increased aerobic glycolysis caused by the addition of D-glutamate without inhibiting the uptake of potassium ions. These investigations suggest that glutamate and aspartate may play a role in regulating cerebral glucose metabolism.

A. Lajtha (New York) reviewed transport mechanisms that control the concentration of amino acids in the brain. Amino acids are transported by a process that requires energy, and mediated transport occurs not only in the uptake but also in the exit of amino acids. The levels are determined by the processes of uptake, exchange, and exit, and any alteration of the physiological level is most likely the result of alterations in uptake or exit rates, which can be individually controlled.

I. Sano (Osaka) reported the isolation and identification of eight new dipeptides from bovine brains, which were the γ-glutamyl peptides of glutamate, glutamine, glycine, β-aminobutyric acid, serine, alanine, valine, and *S*-methylglutathione. From the same laboratory, the isolation and identification of some basic dipeptides from bovine brains, such as 3,7-diaminoheptanoic acid, 2, 2′-aminoethyl-3,7-aminoheptanoic acid, and homocarnosine, were also reported by Y. Kakimoto (Osaka).

Y. Takahashi (Niigata) examined the mechanism of protein biosynthesis in cerebral ribosomal systems. He reported the synthesis of phosphatidyl peptides in microsomal and mitochondrial systems.

S. Ishikawa (Tokyo) reported the

rapid labeling of the phosphoserine moiety of brain phosphoproteins by P^{32}-labeled adenosine triphosphate (ATP) in vitro and presented a scheme for the relation of the soluble and particle-bound phosphoprotein kinases to their respective substrates.

Y. Kawakita (Osaka) compared the physicochemical characteristics of brain microsomes and ribosomes with the respective particles in other tissues. After the molecular size, sedimentation pattern, RNA content, and the base composition of the RNA of the various particulate fractions were determined, the fractions were examined in the electron microscope. Incorporation of P^{32} into various RNA's was measured, with the highest specific activity being found in the nuclear fraction.

O. Hayaishi (Kyoto) investigated the biosynthesis of nicotinamide adenine dinucleotide (NAD) in rat brain with subarachnoidally administered C^{14}-labeled precursors. Nicotinic acid was rapidly converted to NAD, but tryptophan could not be utilized as a precursor because quinolinate transphosphoribosylase is not present.

In sessions on biogenic amines, S. Udenfriend (Bethesda, Maryland) reviewed the biosynthesis of noradrenaline, involving tyrosine hydroxylase, 3,4-dihydroxyphenylalanine decarboxylase, and dopamine-β-hydroxylase. Tyrosine hydroxylase was suggested as a rate-limiting step in the overall synthesis. It was shown that the turnover rates of noradrenaline varied from tissue to tissue and, within a tissue, depend on factors which influence sympathetic activity.

M. Kurokawa (Tokyo) reported on the Na-K-adenosine triphosphatase in nerve-ending particle fractions, obtained by Ficoll density gradient ultracentrifugation. The enzymic activity in the fraction was localized in the synaptosomal limiting membrane rather than in the synaptic vesicles. Such studies show the physiological significance of the synaptosomal membrane, which represents a special type of membrane system characteristic of the nervous tissue. A study on the metabolism of monoamines in pigeon brain was presented by R. Takahashi (Tokyo), who reported the inhomogeneous distribuion of monoamines and their related enzymes. He found a linear correlation of the levels of at least three of four neurohumors in each brain area.

J. Axelrod (Bethesda, Maryland) discussed the uptake, storage, release, and metabolism of catecholamines in the adrenergic nervous system. H^3-noradrenaline is selectively taken up in tissues rich in adrenergic nerves, although this amine hardly crosses the blood-brain barrier. When the sympathetic nerves were denervated, noradrenaline uptake and retention were not observed. Noradrenaline which was taken up was localized in the sympathetic synaptic vesicles. Stimulation of sympathetic nerves caused a release of noradrenaline. When H^3-noradrenaline was introduced into the lateral ventricle, it rapidly mixed with the endogenous brain noradrenaline and was taken up in the synaptosomes. Imipramine and amphetamine blocked this uptake. This neurohumor is stored in an easily releasable and more firmly bound form.

O. Hayaishi described the properties of the particle-bound tryptophan-5-hydroxylase. This enzyme, which with a decarboxylase converts tryptophan to serotonin, is highly concentrated in brain. It is located in the same particle as the decarboxylase, where the product, serotonin, also is stored.

T. Nagatsu (Nagoya) purified mitochondrial monoamine oxidase of brain by the combined use of cutscum and sonication. This made it possible to investigate some of its properties, such as substrate specificity and *p*H optimum, and it was found that it is a metal-dependent sulfhydryl enzyme.

M. Ozaki (Nagoya) studied the metabolism of monoamines in a hypertensive strain of rats. The higher concentrations of serotonin and noradrenaline in the tissues of this strain are due to the lower activity of monoamineoxidase in the liver and to renal dysfunction. This was indicated by the effects of nephrectomy and of adrenalectomy on amine levels.

H. Utena (Maebashi) reported behavior abnormalities and changes in brain constituents in mice and cats during prolonged administration of metamphetamine. The animals showed marked reduction of motor activity. The ammonia content of the brain was decreased and acetylcholine was increased; the amounts of ATP, creatine phosphate, and lactic acid were not changed. Serotonin concentration increased in most brain areas and decreased in the caudate nucleus. The time course of these changes paralleled behavioral aberrations.

E. W. Sutherland (Nashville, Tennessee) described his studies on adenyl cyclase. This enzyme, which catalyzes the formation of cyclic adenylic acid (AMP), is particularly high in mammalian brain. A number of physiological and biochemical actions of catecholamines can be explained by the increased accumulation of cyclic AMP in the tissues. The accumulation is a result of the stimulatory effect of amines on adenyl cyclase. This enzyme in brain is stable in association with particulate material, but is very labile when solubilized. The significance of this enzyme in brain was discussed in relation to membrane permeability and neuronal function. Purification of adenyl cyclase from bacteria was reported by O. Hayaishi who found that, in addition to magnesium, another low-molecular cofactor is essential for enzyme activity.

M. G. Larrabee (Baltimore, Maryland) discussed metabolic changes caused by electric stimulation in excised cervical sympathetic ganglia. Excitation produced increases in oxygen consumption, production of carbon dioxide, glucose utilization, and lactate formation, while lack of glucose or oxygen impaired synaptic transmission. It seems likely that the oxidation of glucose is the main energy source for maintenance of activity in sympathetic ganglia. Electric stimulation also increased incorporation of P^{32} into phosphatidyl inositol; this increase was eliminated by curare, a blocking agent for synaptic transmission.

Y. Nagata (Tokyo) discussed the changes in amino acid metabolism in excised cervical sympathetic ganglia upon electrical stimulation. Absence of γ-amino-butyric acid (GABA) and its related enzymes in the ganglia was indicated, and although certain amino acids accumulated, no GABA uptake could be shown.

O. H. Lowry (St. Louis, Missouri) described his microanalytical methods which make it possible to measure substrate levels and enzyme activities in single nerve cells. By measuring changes in metabolite levels in the first few minutes after death, major control steps in glucose metabolism could be established. These seem to be those which are concerned with the phosphorylation of glycogen, glucose, and fructose-6 phosphate.

M. Satake (Niigata) described his technique for obtaining pure neuronal perikaria by sucrose density gradient centrifugation after gentle homogeni-

zation of the tissue in a solution of sucrose-glycerol-acetone. The isolation made it possible to determine protein, RNA, and DNA content of perikaria. He found a rapid incorporation of C^{14} lysine into nerve-cell proteins.

H. Naruse (Tokyo) discussed the mechanism of carbon dioxide fixation into amino acids and into intermediates of the tricarboxylic acid cycle in nervous tissue. This mechanism might participate in the regulation of carbohydrate metabolism.

S. Hirano (Tokyo) analyzed the mechanism of incorporation of P^{32} from ATP into microsomal proteins. This is the fraction in which Na-K-adenosine triphosphatase is concentrated, and phosphorylation of an unstable intermediate may be a part of the activity of transport adenosine triphosphatase. H. Yoshida (Osaka) reported that calcium binding in brain microsomal and nerve ending particles is accelerated by ATP and magnesium, which suggests a possible role of this reaction in the neuronal excitation-inhibition processes.

J. Axelrod reported the neural regulation of hormonal secretion of the pineal gland. This gland synthesizes and secretes melatonin, which has an inhibitory action on the gonads. The amount of melatonin and the activity of the enzyme which synthesizes it are decreased by continuous light. The effect of light is transmitted to the pineal gland from the eyes by way of the cervical sympathetic nerves. The pineal gland responds to the diurnal change of light and shows the circadian rhythm, being highest at midnight and lowest at noon. Thus the pineal gland may be one of the biological clocks in the body.

R. Takahashi discussed changes in brain ammonia and acetylcholine content caused by electrically induced convulsions in mice. He stressed the close relation between the level of acetylcholine in the brain and the threshold of electric stimulation for convulsions.

O. H. Lowry discussed the major control steps of the various pathways of carbohydrate metabolism in the brain. He analyzed the substrate concentrations and the sequential changes in these concentrations in anoxia. He also discussed mechanisms controlling metabolic rates.

Y. Tsukada reported the incorporation of P^{32} in vitro into highly polymerized RNA in the isolated rabbit retina in which the electrical responses to light stimulation could be recorded. Nuclear RNA was actively labeled in both light and dark conditions although no such incorporation occurred in brain cortex slices. The rapidly labeled RNA was not identical with ribosomal RNA in its sedimentation in sucrose density gradients.

Discussion after formal presentations dealt with the present findings and future possibilities in the relationship between neural function and metabolic regulation in the nervous system. The evidence for the participation of biochemical processes in memory were discussed, and also a possible differentiation between short and long term memory on biochemical grounds.

The topic of the roundtable session was metabolic compartmentation. G. Takagaki, H. Naruse (Tokyo), and A. Lajtha discussed the metabolic compartments of amino acids, especially glutamic acid, in the brain. M. Kurokawa (Tokyo) reported compartments of acetylcholine in brains of normal and epileptogenic mice in the subcellular particles. The discussion centered on the structural basis of these compartments and the compartments of enzymes and substrates. The influence of such compartments on kinetic measurements of metabolic rates was stressed.

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Cerebrovascular Disease

The fifth Princeton Conference on Cerebrovascular Disease was held in Princeton, New Jersey, 5–7 January 1966. Papers were presented by 19 speakers. Clinical and basic scientists, numbering 70, attended the conference and took part in the discussions.

D. Detweiler (University of Pennsylvania) pointed out during a discussion on cerebral atherosclerosis that the most common animal form of this disease can be observed in aged swine. However, there is no correlation between coronary and cerebral atheroscleroses in these animals even though the aorta is always involved.

H. Ratcliffe (Philadelphia Zoological Garden) spoke about ecological factors associated with atherosclerosis in zoo animals. He noted interrelationships between sociosexual environmental factors and atherosclerosis in chickens. It appeared from long-range study at the Philadelphia Zoo that reasonable care had been taken to exclude the effect of diet in the different "population groups" studied.

The fine structure of experimental atherosclersosis, and particularly his own work on the subject, was reviewed by J. Geer (Louisiana State University Medical Center). Geer defined certain of the fine structures he believes to be important in the pathogenesis of atherosclerosis in the laboratory animal. He has observed many of the same findings in early atherosclerosis (intimal streaks in the aorta) in humans.

The Framingham research project was described by W. B. Kannel (Heart Disease Epidemiology Study, Framingham, Massachusetts). During this epidemiologic investigation of cerebrovascular disease 90 case histories were studied. The diagnosis was infarction in 63 percent, subarachnoid hemorrhage in 18 percent, embolism in 15 percent, and hypertensive intracerebral hemorrhage in 4 percent. It was reported that the risk was increased fivefold in persons having hypertension and that definite increase in concentration of cholesterol in the plasma before age 40 appeared to be a "poor risk" factor. When hypercholesteremia, hypertension, and electrocardiographic changes were present simultaneously, the risk of focal cerebrovascular disease appeared to be increased eightfold.

A. Lilienfeld (Johns Hopkins University) raised the question of the significance of the relative contribution that each risk factor (increased plasma cholesterol, changes in electrocardiogram, and hypertension) makes in relation to the other risk factors and their individual or combined relation to the cerebral infarction. He interpreted Kannel's data as suggesting that cerebral infarction is a late-in-life manifestation similar to myocardial infarction. The matter of the significance of transient ischemic attacks as "precursor" events to cerebral infarction independently or in relationship to other risk factors was discussed; no satisfactory answers were presented.

The changing pattern of cerebrovascular disease in the United Kingdom was described by P. O. Yates (University of Manchester). His analysis of death certificates (1932 to 1961) showed