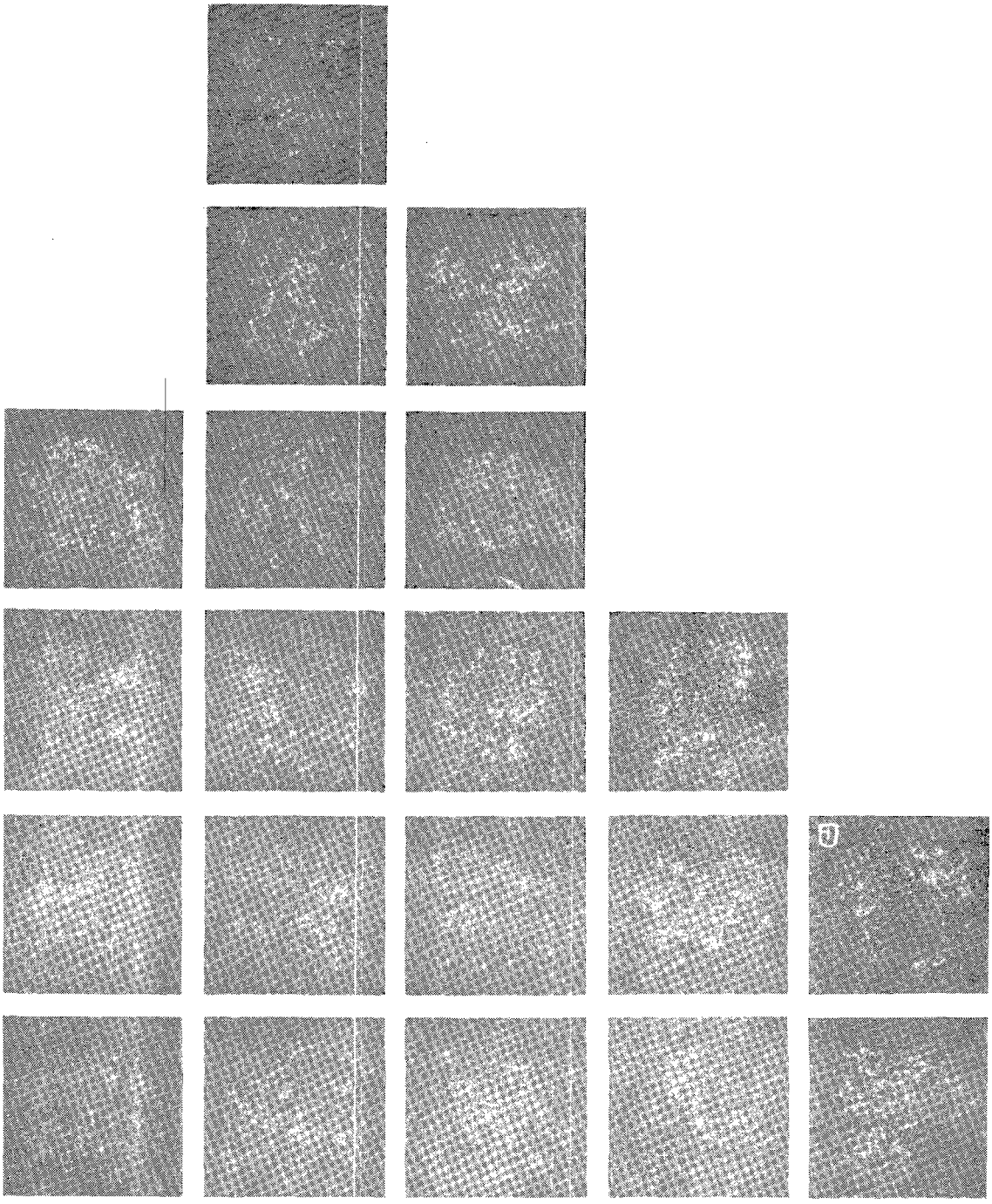
神経化学

VOL. 1 NO. 5 OCTOBER 1962



神 経 化 学 懇 話 会

—— シンポジウム ——

"METHODS OF SEPARATION OF SUBCELLULAR STRUCTURAL COMPONENTS"

A Symposium of the Biochemical Society held on May 11, 1962, at Louvain, Belgium.

Introductory Remarks: the Scope and Limitations of Cell Fractionation. By D. de Duve (*Laboratory of Physiological Chemistry, University of Louvain, Belgium*)

Methods for Disrupting Cells. By D.E. Hughes and V.R. Cunningham (*Medical Research Council Cell Metabolism Research Unit, Oxford, and Medical Research Council Research Group on Demyelinating Diseases, King's College, Newcastle upon Tyne*)

A Comparative Account of Methods for the Isolation of Nuclei. By D.B. Roodyn (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

The Influence of the Composition of the Suspending Medium on the Properties of Mitochondria. By J.B. Chappell and G.D. Greville (*Department of Biochemistry, University of Cambridge, and Biochemistry Department, Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge*)

The Separation of Subcellular Structures from Brain Tissue. By V.P. Whittaker (*Biochemistry Department, Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge*)

The Significance of Subcellular Fractionation as Applied to Certain Hydrolytic Enzymes. By J. Conchie and G.A. Levvy (*Rowett Research Institute, Bucksburn, Aberdeen, Scotland*)

Techniques for the Isolation of Particulate Glycogen and its Examination in the Electron Microscope. By P. Drochmans (*Laboratoire de Cytologie et de Cancerologie Experimentale, Université Libre de Bruxelles*)

The Separation of Ribosomes and Oxidosomes from Bacteria. By J. de Ley (*Laboratory for Microbiology, Faculty of Sciences, State University, Ghent, Belgium*)

Medium Composition and Equilibrium Density of Subcellular Particles from Rat Liver. By H. Beaufay (*Laboratory of Physiological Chemistry, University of Louvain, Belgium*)

以上は Biochem. J. 84(1) July, 1962. の記録からうつしとつたものである. いづれ Biochemical Society Symposia の 1 つとして本になるものであろう. 最も関心が深いと思われる Whittaker の抄録をそのまま次にかゝげる. 我々の懇話会のシンポジウムの話題のたねとして頂きたい. (編集部)

The Separation of Subcellular Structures from Brain Tissue

By V.P. Whittaker. (*Biochemistry Department, Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge*)

Electron-micrographic studies made recently by Dr. E.G. Gray, and briefly reported by Gray & Whittaker (1962a), show that when slices of brain cortex are soaked in 0.32 M-sucrose at O° for 30 min. five characteristic structural changes take place. These are (1) a large increase in extracellular space, (2) swelling and disintegration of dendrites and cell bodies of neurones, (3) condensation of mitochondrial membranes with vesication and a 'zebra striped' appearance of the cristae, (4) spontaneous formation of microsomes in the cytoplasm, (5) the shrinking and pulling away of nerve endings from their axon terminations and post-synaptic attachments. The response of the tissue is consistent with the dissolution of intercellular cement, perhaps by chelation of calcium ions by sucrose, the penetration of sucrose and water into cell bodies and dendrites and an ensuing transfer of water from the nerve endings to the cell bodies. Similar but less extensive effects are seen after 5 min. It would be interesting to know if other carbohydrates produce similar effects, especially inulin, which has been used to measure extracellular space. The changes induced by sucrose are in marked contrast with the effect of soaking in isotonic saline, which is simply to cause some swelling of the tissue without the separation and disintegration of cell membranes seen in sucrose.

Observations of the torn edges of tissue sections (Gray, 1959) show that the dendrites and surrounding glial processes are also mechanically less robust than nerve endings and tend to disintegrate under shear forces leaving the nerve endings intact. A combination of the effects of sucrose and mechanical shear could therefore account for the way in which nervous tissue breaks down when homogenized in sucrose. In addition to the release of nuclei and mitochondria, and the formation of microsomes and myelin fragments, nerve endings are torn or pinched off their attachment (Gray & Whittaker, 1960, 1962b) to form isolated neve-ending particles (NEP's) about 1 μ in diameter with similar sedimentation characteristics in 0.32 M-sucrose to mitochondria. These particles appear as thin-walled bags packed with synaptic vesicles, the characteristic cytoplasmic organelles of the nerve ending about 500 Å in diameter; they also often contain one or more small mitochondria and may, in addition,

have a short, thickened length of post-synaptic membrane still adhering to their periphery. The various particles formed by homogenization overlap in size, so that differential centrifugation alone cannot separate them. However, by combining this with density-gradient separation, nuclei, myelin, NEP's, mitochondria and microsomes can all be separated as distinct and relatively homogeneous fractions in good yield.

The isolation of NEP's opens up the possibility of a detailed study of the biochemistry of synaptic function, and recent progress along these lines will be described. By washing the particles repeatedly with distilled water, a membrane preparation can be obtained which is apparenlty free from cytoplasmic constituents and which is probably the purest preparation so far available of nerve membranes. Attempts have also been made to prepare isolated synaptic vesicles. Osmicated vesicles are released, together with fragments of osmicated cell membranes, if osmicated NEP's are subjected to gentle mechanical disintegration during dehydration (Whittaker, 1959; Gray & Whittaker, 1962b). In the native state, however, they remain clumped together as though embedded in a jelly-like cytoplasm, even when the outer membrane of the NEP is disrupted. It is thus doubtful whether the vesicles isolated by de Robertis, de Iraldi, Rodriguez & Gomez (1961) in the microsome fraction are in reality synaptic vesicles.

The isolation of NEP's demonstrates that subcellular fractionation techniques may be used to isolate not only intracellular organelles as such, but also functional portions of cells. Another illustration is the ingenious magnetic separation of renal glomerulae after iron embolism (Cook & Pickering, 1959). This approach to the study of cellular function will increasingly require close morphological control, usually at the electron-microscopic level, of the material being isolated. Unfortunately the conventional histological techniques for the electron microscope are extremely time-consuming; thus a biochemist may produce in one day enough material to keep an electron microscopist busy for weeks. We have therefore attempted (Horne & Whittaker, 1962) to adapt for the study of mammalian subcellular fractions the negative-contrast (negative-staining) method so successfully used for the study of viruses and bacterial cells (Horne, 1961). In this method, particulate material is simply mixed with 2% phosphotungstic acid (PTA), previously neutralized to pH 7.4 and sprayed on to specimen grids with an atomizer which produces fine droplets of 1–10 μ in diameter. Under favourable conditions, the PTA forms an electron-dense glass in which the unstained particles show up as areas of low electron density. The particles are seen from above and not in section, and therefore may have

an unfamiliar appearance; however, we have been able to identify all the main constituents of brain homogenates. Electron micrographs obtained by this method suggest that synaptic vesicles are distinct subcellular organelles, and resemble red cells in shape rather than ping-pong balls; they also confirm that disruptive treatments do not release the vesicles and suggest that in some cases the effect of the disruptive treatment may simply be to cause the vesicles to fuse with the outer membrane. In work on membrane fractions the method has also proved extremely useful as a rapid check on the homogeneity of preparations after subfractionation. Some membrane structures not previously seen in homogenates have been revealed, notably large sheets of endoplasmic reticulum studded with ribosomes. The method may be used on fresh, formalin-fixed or osmicated particles; formalin-fixation is particularly useful for preserving mitochondria. These are often long, horseshoe-shaped and waisted as though in process of budding off. The large amount of morphological detail obtainable by the new technique will undoubtedly complement that obtained by thin sectioning, and its inherently greater resolving power can be expected to furnish important new results.

The negative-contrast technique cannot as yet be applied to tissues. The survival in brain homogenates of discrete portions of cells provides material which enables it to be applied for the first time to organized cytoplasm.

Cook, W.F. & Pickering, G.W. (1959). The location of renin in the rabbit kidney. J. Physiol. **149**, 526–536.

de Robertis, E., de Iraldi, A.P., Rodriguez, G. & Gomez, J. (1961). On the isolation of nerve endings and synaptic vesicles. J. biophys. biochem. Cytol. **9**, 229–235.

Gray, E.G. (1959). Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. J. Anat., Lond. **93**, 420–433.

Gray, E.G. & Whittaker, V.P. (1960). The isolation of synaptic vesicles from the central nervous system. J. Physiol. **153**, 35–37P.

Gray, E.G. & Whittaker, V.P.(1962a). On the synapse: biology and morphology. Brit. med. Bull. **18**(3) -in the press-

Gray, E.G. & Whittaker, V.P. (1962b). The isolation of neve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. J. Anat., Lond. **96**, 79–88.

Horne, R.W. (1961). In *Techniques for electron microscopy*. Ed. by Kay, D.D. Oxford: Blackwell Scientific Publications.

Horne, R.W. & Whittaker, V.P. (1962). Z. Zellforsch. -in the press-

Whittaker, V.P. (1959). The isolation and characterization of acetylcholine containing particles from brain. Biochem. J. **72**, 694-706.

因みに Whittaker, V.P. の関連の論文を列記すると次の如くである. (編集部)

Intracellular distributions of acetylcholine and choline acetylase (Hebb C.O. & Whittaker V.P.) J. Physiol. **142**, 187-196, 1957

The isolation and characterization of acetylcholine containing particles from brain (Whittaker V.P.) Biochem. J. **72**, 694-705, 1959

The isolation of synaptic vesicles from the central nervous system (Gray E.G. & Whittaker V.P.) J. Physiol. **153**, 35-37P, 1960

The binding of neurohormones by subcellular particles of brain tissue (Whittaker V.P.) *Regional neurochemistry*, 259-263, ed. Kety S.S. & Elkes J. Oxford: Pergamon Press, 1961

The subcellular localization of transmitter substances in the central nervous system (Whittaker V.P.) Biochem. Pharmacol. **5**, 392-398, 1961

The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation (Gray E.G. & Whittaker V.P.) J. Anat., Lond. **96**, 79-88, 1962

The distribtion of hydroxytryptamine in brain fractions (Michaelson I.A. & Whittaker V.P.) Biochem. Pharmacol. **11**, 505-506, 1962

The subcellular distribution of histamine in guinea-pig brain (Michaelson I.A. & Whittaker V.P.) Biochem. J. **84**, 31P, 1962

On the synapse: biology and morphology (Gray E.G. & Whittaker V.P.) Brit. med. Bull. **18**(3), 1962

"THE CYTOLOGY OF NERVOUS TISSUE"

A Symposium of the Anatomical Society of Great Britain and Ireland held in November, 1961.

SECTION 1. ENZYMES AND HISTOCHEMISTRY

R. Holmes. Esterases of the hypothalamo-hypophysial portal system.

P.R. Lewis and C.C.D. Shute. Intra-axonal localization of acetylcholinesterase in normal brains and distributional changes following tractotomy.

R.S. Snell. Demonstration of neurones using the histochemical technique for cholinesterase.

T.H. Williams. Some aspects of kidney 'neurility' as studied in the cat.

N. Cauna. The submicroscopical relationship between the nervous and non-nervous elements of the cutaneous receptor organs and its significance.

A. Peters. Anatomical considerations of the site of the blood-brain barrier.

I. Smart. Neuroglia—an example of the evolution of transport cells in tissues with reduced intercellular space.

R.E. Coupland. Histochemical observations on the distribution of nerve fibres in adrenal glands of the ox and man and in a human phaeochromocytoma.

T.A. Quilliam and J. Armstrong. Structural and denervation studies of the Herbst corpuscle.

G. Horn. A versatile, inexpensive, stereotaxic instrument for use with monkeys and cats.

SECTION 2. DEGENERATION AND REGENERATION

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P.R. Gould and S.J. Holt. Observations on the participation of acid phosphatase and esterase in rat sciatic nerve undergoing Wallerian Wallerian degeneration.

E. Palmer, R.J.W. Rees and G. Weddnell. The phagocytic activity of Schwann cells.

H.J. Gamble. Endoneurial mast cells and their reaction in nerve degeneration.

Catherine Hebb. Choline acetylase in regenerating somatic nerves.

W.M. Cowan and T.P.S. Powell. Some observations on the use of on-the-slide paraffin methods for studying fibre degeneration in the central nervous system.

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E. Beck and P.M. Daniel. Degeneration and regeneration in the hypothalamus.

R.W. Guillery. Fibre degeneration in the efferent mamillary tracts of the cat.
T.P.S. Powell. Transneuronal cell degeneration in the auditory relay nuclei.

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O. Perier and E. de Harven. Electron microscopy of cultures of central nervous system.

S.L. Palay. The electron microscopy of the glomeruli cerebellosi.

H. Gansler. Electron microscopy of cat diencephalon and neurohypophysis.

A.D. Dixon. Cytology of the trigeminal ganglion.

M. Kidd. Electron microscopy of synapses in the inner plexiform layer of the retina.

N. Dilly. A comparison of the cytoplasmic organization of glial and neuronal connections in the goldfish central nervous system.

—— 神経化学文献 ——

凡 例

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II. Active transport (Wilbrandt W.)—Possible mechanisms of active transport (Wilbrandt W.)—Effects of drugs on active transport (Beyer K.H. & Baer J.E.)

III. Multiple mechanisms (Gaddum J.H.)—Action of barbiturates upon respiratory enzymes (Aldridge W.N.)—Appraising enzymic actions of central depressants by examining cerebral tissues (McIlwain H.)

IV. Receptors (Paton W.D.M.)—Relation between enzymes and cholinergic receptors (Waser P.G.)

V. Altered drug metabolism (Williams R.T.)

VI. Drug metabolism: subcellular aspects (Peters R.)—Drug metabolism, subcellular mechanisms (Brodie B.B.)

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—— 会員の交流 ——

スライスをつくるためのさゝやかな工夫：台弘（群大精神），金松直也（信大精神）

マウスの脳のような小さな材料からスライスを手際よくつくるには苦労がいる．この場合には皮質の表面に平行なスライスはとれないから，表面に垂直な切り方をする．まず半球を裏返しにして丁寧に白質をとる．

次に発泡スチール（スチロフォーム）の小ブロックをまな板にして—これが工夫である—表面を浮遊液で濡らし，半球を軟膜面を上にして載せ平らに拡げる．スチロフォームは近頃よく包装用に使われているし，また2尺角1～2寸厚さの板としても売っている．それをナイフでタバコの箱位の大きさに切りとつて用いる．組織や水はスチロフォームになじまない．

安全カミソリの刃でフリーハンドに組織から切片をきるのだが，この時刃をそのまゝ土台のスチロフォームに切り込んで立てゝおき—これが味噌である—，濡した筆先でおもむろに切片をとつて液溜に移すことができる．スライスを切る時に，片手に楔形に切つたスチロフォーム片をもつて，楔の先の彈力で組織を上から軽くおさえておくと，組織は全く動かないから都合がよい．スライスを移したら刃を抜いて次のを切る．こうして1ケ数mgの切片ができる．

秤量には，2.0× 1.5cm位のパラフィン・フイルム（パラフイルム）を用意し—これも工夫である—風袋を秤つておき（約50mg），液溜からスライスを細いガラス棒の曲つた先でひき上げてパラフイルム片の上にならべる．8—10ケ位で50～60mgとなる．短冊に切つた小濾紙片のヘリをスライスに近づけて水分をのぞく．パラフインも組織や水になじまないから処理し易い．風袋ごと秤量して差引き，組織の湿重量を求める．1匹の脳から合計 150mgの切片がらくにとれる．

些細なことだが，お役に立つことがあれば幸甚である．

—— ニュース ——

第3回　常任委員会議事録

9月30日（日）大阪に於て会長中脩三教授を加え，第3回常任委員会を開催した．主なる議事は次の如くで，具体的事項は11月18日の総会において議決して頂く予定である．

（1）　第5回神経化学懇話会プログラム編成．応募原稿が意外に多数であり，2日間の会期で消化することは不可能であるとの理由で，シンポジウムに重点をおき，活発な討論の期待出来ない申込演題については，今回はご遠慮ねがうこととし，プログラムを編成した．懇話会の主旨にもとづき，やむをえないことであつたが，常任委員会一致で決定をみた．

（2）　会費値上げ案．今年度の会員数・会費・会計報告を考慮の上，郵送料の値上りなどに伴い，来年度（昭和38年）より会費年額1000円とする案を可決した．

（3）　委員追加．若干の方々に新委員として加つて頂くよう協議し，候補案を決定した．

（4）　来年度講演会．来年は医学会総会など外国学者の来日が多く予定されているので，随時機会をとらえ，講演会を開催する計画を協議した．

（5）　会誌の件．現在の会誌の内容は変則的なものであるので，会員の交流，綜説などの欄を設け，一般学会誌としての体裁をととのえる方向に努めることを申し合せた．

（6）　来年度懇話会の開催．10月初旬群大台弘教授会長のもとに東京で開催する案を可決した．

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(昭和37年度)

— 9 月 17 日現在 —

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