like pyrogallol were converted into brown or red-brown products rapidly.

The catalytic activity of the gel was markedly reduced by 0·01–01 m Al(III), Ca(II) and Mg(II). Hydroquinone, resorcinol, and halgenated monophenols also inhibited pyrogallol oxidation on the gel. Methylene blue which is strongly adsorbed by silicagel also seemed inhibitory whereas eosin which has little affinity for silicagel also failed to suppress its catalytic activity.

Discussion. The experiments which have been described demonstrate the silicagel activated oxidation of *o*-diphenols. Accordingly, they provide evidence for the formation of complexes between silicagel and these phenols, as do earlier investigations cited.

The importance of hydrogel structure and surface charge, in catalysis are indicated by heat inactivation, the inactivity of β-quartz, and the inhibitory effects of cations. Phenolic inhibitors suggest that hydrogen bonding may also be one of the significant interactions between substrate and catalyst.

The specificity of silicagel activation (as distinct from adsorption alone) for *o*-diphenols parallels the silicate-coordinating properties of these compounds. In both systems catechol, pyrogallol and gallic acid were active, whereas phenol, hydroquinone, and resorcinol were not. Although some inorganic material is carried into solution the conditions described in this report clearly do not favor complete or extensive silicon-oxygen bond scission. Nevertheless it is likely that catalysis involves the coordination of *o*-diphenols at the silicagel surface. The oxidation products are in part firmly adsorbed on the gel, but are released by acid, perhaps by decomposition of surface complexes.

These findings support the phenol-mineral interactions and mineral-catalyzed phenol oxidations[7], which have been proposed as factors in the formation of humic substances in soil.

S. M. Siegel

Union Carbide Research Institute, White Plains (N. Y.), May 30, 1960.

Zusammenfassung

Die Oxydation von Pyrogallol, Brenzkatechin, Gallussäure und Dopa wird von Silikagel, nicht jedoch von β-Quarz, katalysiert. Diese Katalyse erfolgt nicht nur in Wasser, sondern auch in wässerigem Alkohol und Chloroform. Dabei scheinen die elektronegativen Oberflächen des Silikagels von Bedeutung zu sein. Mg^{2}, Ca^{2+} und Al^{3+} sowie verschiedene Phenole, die keine *o*-Dihydroxygruppierung besitzen, hemmen die katalytische Oxydation.

Von den geprüften Phenolen sind nur bei *o*-Diphenolen Komplexbildung mit Si^{4+} und katalytische Oxydation durch Silikagel festzustellen.

Die Bedeutung von Phenol-Mineral-Komplexen bei der Bildung von Humusstoffen wird durch die vorliegenden Untersuchungen gestützt.

A Differential Staining Reaction Demonstrating Reciprocal Activity in Mauthner's Cells[1]

In an attempt to relate the structure of the neuron with its function, extensive neurohistological and neurophysiological studies are being made on the bilaterally represented Mauthner's cells which lie in the floor of the medulla oblongata of teleosts. It was proposed[2,3], that these 2 cells function as reciprocating units so that while one cell is actively excited the other is actively inhibited. This functional relationship fulfills the requirements of a system demonstrating reciprocal inhibition of motor neurons by the sensory afferents.

It was also noted[2] that in some histological preparations of the Mauthner's cells of the bullhead there was a differential staining of the various parts of these neurons (dendrites, cell body, and axon hillock region). It was suggested that this staining reaction might be indicative of specific intracellular changes characterizing neuronal excitation as well as inhibition.

This report is concerned with a further attempt to determine the effects of controlled stimulation of the VIIIth nerve afferents on the staining reaction of the paired Mauthner's cells of the bullhead.

Materials and Methods. Fifteen bullheads (10 experimental and 5 controls) were used. Under hypothermic anesthesia, craniotomy exposed the VIIIth nerve roots and facilitated rapid removal of the brain for processing by freeze-dehydration. In the experimental fish the entering roots of the VIIIth nerve were stimulated at threshold level (1 sec at 10/sec). This was done on the right side of 5 fish and on the left side of the other 5. The brain was removed immediately and processed. The same procedure was followed on the 5 control specimens except that no stimulus was applied to the nerve roots.

Tissues frozen in Isopentane (– 100°C) are dehydrated for 7 h in vacuum apparatus at pressure of not more than 1·0 μ and temperature – 40°C. Dehydrated tissues embedded in paraffin are sectioned serially and mounted on glass slides. Staining is similar to the classical Bodian protargol technique[4] except reduction is carried out using the Pearson-O'Neill silver gelatin[5]. Deparaffinized sections, after hydration in 3 changes distilled water, 2 min each, are placed in freshly prepared 0·5% aqueous protargol with a 2×2 inch copper plate in the glass container. Incubate in 37°C oven 18–24 h. Wash 10 min in running water, 2 changes of distilled water, 5 min each, and one change of buffered[6] water for 2 min. Reduce in silver gelatin[7] mixture for 30 sec. Wash in 4 changes of distilled water, 5 min each. Tone in 0·2% gold chloride 2 min. Wash in 2 changes distilled water, 5 min each. Place in 2% oxalic acid for 15 sec, wash in 2 changes distilled water, 5 min each, and finally treat in 5% sodium thiosulfate, 2 changes, 5 min each. Wash in running water for 5 min, dehydrate and cover.

Results. The typical staining reaction of the Mauthner's cell on the same side as the applied VIIIth nerve stimulus is characterized by dendrites and perikaryon which are colored a deep purple while the axon hillock and the proximal part of the axon is an almost translucent lavender (Fig. 1). In contrast, the Mauthner's cell contralateral to the applied stimulus stains in a reverse manner with the dendrites and cell body colored lavender and the axonal

[1] Supported in part by the NIH Grants MY-3271 and H-3084.

[2] E. Retzlaff, J. comp. Neur. *101*, 407 (1954).

[3] E. Retzlaff, J. comp. Neur. *107*, 209 (1957).

[4] D. Bodian, J. comp. Neur. *68*, 117 (1937).

[5] A. Pearson and S. L. O'Neill, Anat. Rec. *95*, 297 (1946).

[6] Buffered water is prepared as follows: 17 ml of 5 *M* acetic acid (12 ml to 1000 ml distilled water) added to 3 ml of sodium acetate (16 g to 1000 ml distilled water). Buffered water prepared by adding 14 ml of stock buffer to 1000 ml distilled water. The pH is approximately 4.1.

[7] The silver gelatin reducing solution, using buffered water, is prepared as follows: (1) 400 ml 3% gelatin heated to 70°C; (2) 100 ml 2% $AgNO_3$; (3) 40 ml 1% hydroquinone. Stir (2) and (3) together and add to (1) immediately before reducing sections.

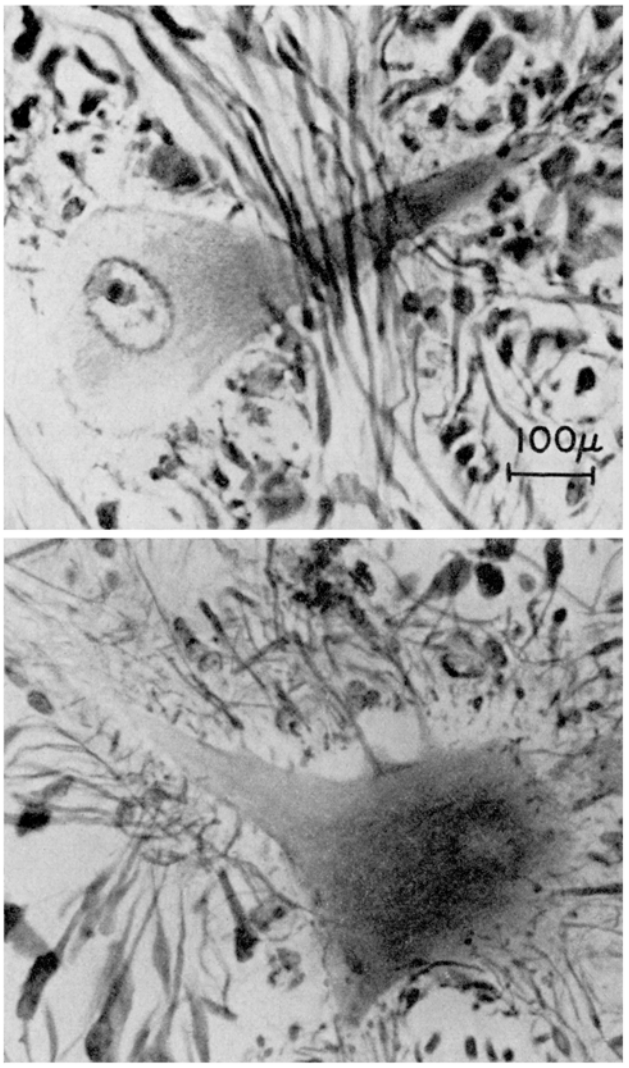

Fig. 1. Photomicrograph of bilaterally represented Mauthner's cells, showing effects of unilateral stimulation of VIIIth nerve root. Cell on the side of the applied stimulus (upper) shows deeply stained dendrites and cell body and a lightly colored axonal region. Dendrites and cell body of the cell contralateral to the applied stimulus stain a light color while axonal area is colored a deep purple. Axon of each cell is directed toward the center and somewhat upward.

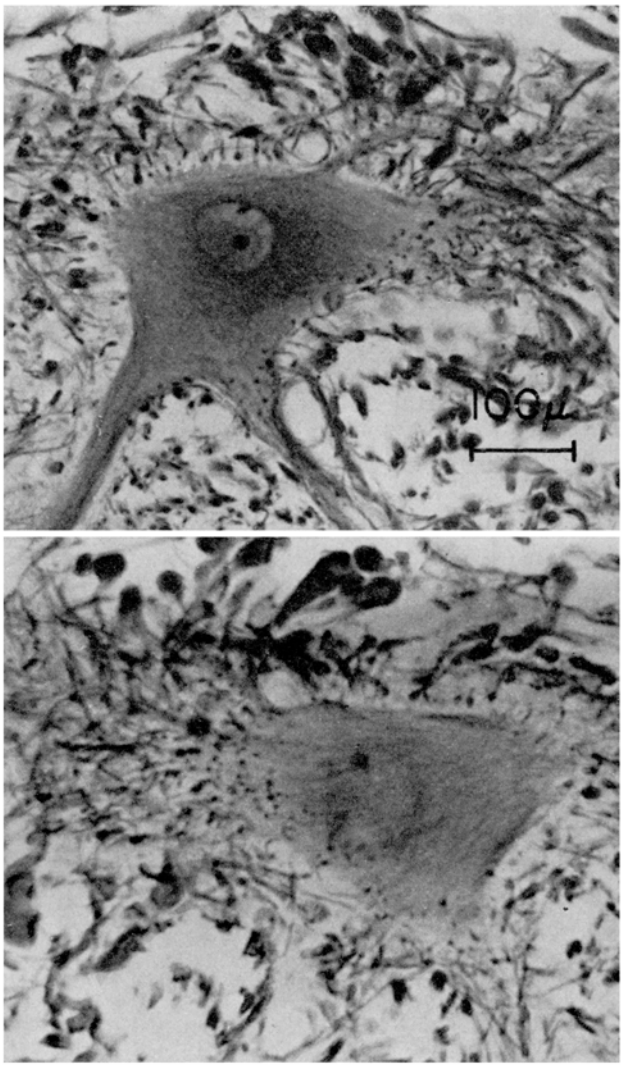

Fig. 2. Photomicrograph of Mauthner's cells in control bullhead. Note uniformity of staining reaction of dendrites, cell body, and axonal region in contrast to staining in the experimental fish. Orientation is the same as in Fig. 1.

pole of the cell a deep purple. Both of the Mauthner's cells in the non-stimulated fish have a rather homogenous lavender coloration (Fig. 2). In all specimens, both experimental and controls, the neuronal elements such as the nucleus appear essentially normal. The several types of boutons terminaux which literally cover these cells are a deep purple color with no indication of any differential staining either in the experimental or control bullheads.

Discussion and Conclusions. VIIIth nerve afferent fibers make synaptic contact with specific parts of the paired Mauthner's cells. There are unbranched VIIIth nerve afferents which end exclusively on the distal part of the lateral dendrite and those which cross the midline to end on the axon pole of the contralateral neuron. Other VIIIth nerve afferents branch in such a way that their synaptic boutons contact the proximal part of the lateral dendrite and the perikaryon of the homolateral cell after which they continue to the opposite side where they end on the axon hillock of the contralateral Mauthner's cell. These two giant neurons are connected by axon collaterals which arise from the axon of one Mauthner's cell and terminate on the axon hillock of the other cell[8].

These structural relationships support the concept of polar function of the neuron[2,3,9]. According to this hypothesis, the afferent fiber terminal boutons on the dendrites and cell body function to excite the neuron, while those on the axon hillock and the proximal part of the axon are inhibitory to neuronal discharge. Thus, the effect of unilateral VIIIth nerve root stimulation would be to excite the homolateral Mauthner's cell. The axo-axonal collaterals then could act as a delaying feed-back loop which would augment the inhibitory effect on the already inhibited neuron.

[8] E. RETZLAFF and JOAN FONTAINE, Science *131*, 104 (1960).
[9] R. GESELL, Ergebn. Physiol. *43*, 477 (1940).
[10] G. J. ROMANES, J. Anat. *84*, 104 (1950).
[11] Special attention is called to *Neuronal Dye-Sorption as a Histochemical Indicator of Nervous Activity* by R. FISCHER and W. ZEMAN, Nature *183*, 1337 (1959).

Unilateral VIIIth nerve stimulation excites only the homolateral Mauthner's cell[3]. Bilateral simultaneous stimulation of the VIIIth nerve does not induce both Mauthner's cells to discharge at the same instant, as indicated by evoked potentials recorded from the Mauthner's axons in the spinal cord[3]. This supports the concept that these two motor neurons are reciprocally affected by VIIIth nerve stimulation. This phenomenon has been designated as simultaneous excitation of one neuron and inhibition of the other by afferent nerve stimulation.

The differential staining reaction suggests an intracellular chemical correlation with functional neuronal excitation and inhibition. The synaptic effect on these two cells may alter the intracellular Cl^- ion concentration in various parts of the neuron, which, in turn, determines the staining reaction of neurons by reduced silver[8,10]. Also, it may be that redox potential changes occur which cause the difference in silver deposition.

Inasmuch as the staining reaction of the two Mauthner's cells is altered by selective stimulation, it is postulated that the dendrites, the cell body, and the axon hillock region are of prime functional importance in nervous integration. The various synaptic endings which display a homogenous staining reaction may serve as activators for the intracellular activity rather than specific excitors or inhibitors[11].

E. Retzlaff and Joan Fontaine

Research Division, Columbus Psychiatric Institute, Ohio State University, Columbus (Ohio), March 29, 1960.

Zusammenfassung

Die Mautnerzellen des Welses (*Ameirus*) zeigen nach Reizung des N. statoacusticus ein färberisches Verhalten, das erlaubt, sie von ungereizten Zellen mit Sicherheit zu unterscheiden.

Quantitativer Nachweis eines Abbaus von Zellulose und Lignin in der rollkranken Rebe

Im vergangenen Jahr wurde mitgeteilt, dass ein spezielles Virus die weitverbreitete Rollkrankheit der Rebe verursacht[1]. Sekundär bewirkt es, dass die rollkranke Vitis Zellulose und Lignin anomal abbaut, wodurch die unverkennbaren Symptome (eingerollte Blätter und durchhängende Triebe) entstehen. Als spezifisches intermediäres Stoffwechselprodukt konnte ich 4-Methyl-D-glukuronsäure (4-M-D-g)[2] erfassen. Diese irreguläre Verbindung kommt in gesunden oder anders infizierten Weinstöcken nicht vor.

Bei schwer rollkranken Pflanzen fiel auf, dass der native Gehalt an Zellulose bei pathologisch gezeichneten Blättern und weichen Trieben auf 0,3% des normalen herabgesunken und Lignin vollkommen verschwunden war. Bei diesen stark zurückgefallenen Reben ergab die partielle Analyse der Kohlehydrate von den grünen Sprossorganen zu 54,7% 4-M-D-g. Leichter kranke, noch fast symptomlose Stöcke enthielten 22% Zellulose, 13% Lignin und 26,3% 4-M-D-g.

Da dieser charakteristische Zerfall der natürlichen Gerüstsubstanz offensichtlich mit dem progressiven Grad der Infektion fortschreitet, versuchte ich ausserdem, den eigentümlichen Metabolismus exakter stufenweise experimentell festzuhalten. Dazu wurden zehn einwandfreie Edelreiser gleichzeitig auf zehn rollkranke Unterlagen gepfropft. Ab 15. Tag nach ausgeführter Kopulation wurde in regelmässigen Abständen von sieben Tagen von den angesteckten, latent kranken Edelreisern je ein äusserlich unverändertes Blatt abgeschnitten und die drei fraglichen Inhaltsstoffe quantitativ chromatographisch bestimmt. Die Einzelergebnisse wurden einer Varianzanalyse nach Fisher und Yates[3] unterworfen. Der mittlere Fehler beträgt $\pm$ 3%. Die Mittelwerte sind mit $P < 0{,}1$ gesichert. Die in der Tabelle zusammengestellten Daten bestätigen, dass mit zunehmender Dauer des virösen Befalls die pflanzlichen Festigungselemente schrittweise reduziert werden und damit verbunden 4-M-D-g kontinuierlich ansteigt.

Tage nach der Inokulation	Gehalt an Zellulose in % der gesunden Kontrolle (= 100)	Gehalt an Lignin in % der gesunden Kontrolle (= 100)	Gehalt an 4-M-D-g in % der gesunden Kontrolle (= 0)
15	90,5	83,4	0,3
22	84,2	80,2	0,48
29	80,7	78,8	0,62
36	76,3	77,5	0,85
41	74,6	75,2	1,4
48	73,4	74,5	1.8
Leichtkranke Reben ohne Symptome. Infektionsdatum unbekannt	22	13	26,3
Schwerkranke Reben mit Symptomen. Infektionsdatum unbekannt	0,3	0	54,7

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Gertrud Ochs

Botanisches Institut der Universität Freiburg (Breisgau), 5. April 1960.

Summary

In leafroll-diseased grapevines, lignine and cellulose decompose anomalously. Therefore leaves and shoots of these vines curl characteristically down and inward. As a specific intermediate product, 4-methyl-D-glucuronic acid has been identified. The decomposition increases proportionally to the progress of the infection.

[1] Gertrud Ochs, Exper. *15*, 303 (1959).

[2] B. Flaschenträger, *Lehrbuch der physiologischen Chemie* (Springer, Berlin 1951), p. 308.

[3] R. A. Fischer und F. Yates, *Statistical Tables* (Oliver & Boyd, London 1949).

Aspetti del nucleolo di cellule normali e patologiche[1]

Nel nucleolo unico degli ovociti di alcune specie di Molluschi (*Aplysia depilans, Mytilus galloprovincialis*) e di Echinodermi (*Paracentrotus lividus, Arbacia lixula*) è stato possibile rilevare che, durante il corso dell'accrescimento ovocitario, in relazione alla rarefazione delle ribonucleoproteine, ed al passaggio da uno stato più denso ad uno meno denso delle sostanze nucleolari, si rendono manifesti

[1] Ricerche effettuate con un contributo del Consiglio Nazionale delle Ricerche Italiano.