Steroids and Brain Edema

Edited by

H. J. Reulen · K. Schürmann

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With 60 Figures

Springer-Verlag
Berlin · Heidelberg · New York 1972

Proceedings of an International Workshop held in Mainz, W. Germany, June 19 to 21, 1972

Sponsor: Sharp & Dohme GmbH., München, subsidiary of Merck & Co., Inc., Rayway, NJ/USA

ISBN 3-540-05958-X Springer-Verlag Berlin · Heidelberg · New York
ISBN 0-387-05958-X Springer-Verlag New York · Heidelberg · Berlin

Typesetting, printing and binding: Universitätsdruckerei Mainz GmbH

Preface

The control of brain edema is still one of the major problems in surgical and conservative treatment of various cerebral lesions. Many attempts have been made to develop methods for reducing the high mortality associated with brain edema. After many years of using hypertonic solutions it can be stated that this type of therapy has not yielded satisfactory results. During recent years increasing evidence has been accumulated on the efficacy of steroids on brain edema. Steroids were reported to result in rapid relief of signs and symptoms of increased intracranial pressure and neurological dysfunction accompanying cerebral edema.

It was the aim of this workshop to evaluate the effect of corticosteroids on brain edema as an advance in therapy, It was hoped that this could be achieved by a multidisciplinary approach. Though, the volume contains the contributions of various experts – internists, neurochemists, neurologists, neuropathologists, neurosurgeons, pharmacologists, physiologists – who have added considerable experimental and clinical evidence on the action of steroids on brain edema. New pathophysiological aspects regarding the mechanisms underlying the formation and resolution of brain edema are presented. The effectiveness of corticosteroid therapy in various forms of clinical and experimental brain edema, e.g. accompanying brain tumors, head injury, spinal cord injury, cerebrovascular lesions, etc. as well as dosage and duration of treatment are critically discussed.

Workshops, although eminently suited to explore specific problems and to interpret the value of new data, have the disadvantage that the information remains confined to a small group. This book, therefore, is thought to be a vehicle for the spreading of this information to all persons interested in the subject clinically or experimentally.

The rapid publication of the proceedings could be realized by the excellent cooperation of the authors, the editorial board, and the Springer-Verlag. We take great pleasure to express our thanks to their invaluable help. Dr. William Meinert helped in compiling and revising of the discussions. We also wish to thank for the generosity of the sponsor, Sharp & Dohme GmbH, München, who supplied the financial basis for the meeting and especially Dr. Helmut F. Hofmann and his staff for their support in the organization of the symposium and their constant attention to our many requests.

Mainz, Dezember 1972

Hans J. Reulen · Kurt Schürmann

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Chapter I

Experimental Aspects of Brain Edema and Therapeutic Approach

Pathophysiological Aspects of Brain Edema

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The subject of brain edema (BE) has been burdened for many years by confusion with regard to understanding the essential pathophysiological aspects of the process. Various classifications were based mainly either on some gross characteristics, such as Reichardt's [29] Hirnödem v. Hirnschwellung, or on etiological factors involved (e.g. traumatic, inflammatory, necrotic, hemorrhagic, etc.), which contributed only some limited descriptive value.

Accepting the definition of edema as an ***abnormal accumulation of a fluid in a tissue*** it follows that there are basically two possibilities for location of such a fluid i.e. it can be intra- or extracellular and in general pathology one finds examples of such basic type of edema according to intra- or extracellular location of edema fluid. Pursuing further this basic division of edema into two types, one can postulate that in intracellular edema the crucial pathogenic event is related to a disturbance in cell membrane permeability of individual cells resulting in cellular imbibition, whereby in the second type an increased vascular permeability allows an increased passage of fluid of hematogenous origin leading to extracellular inundation of the tissue.

Assuming that a similar situation must also exist in the brain, I proposed the terms *vasogenic* and *cytoxic* types of BE as describing best two basic different pathomechanisms which may be involved in abnormal fluid accumulation in the brain [14]. At that time there were still forthcoming electron microscopic (EM) reports claiming that BE is always intracellular and that large extracellular spaces occasionally observed were created by rupture of cell membrane, either *in vivo* or during EM processing. This misconception persisted until a sizeable, functional extracellular space allowing a free diffusion of even large protein molecules in the normal brain, as well as, an extracellular spread of edema fluid deriving from injured blood vessels have been unequivocally demonstrated in numerous EM studies [4, 6, 9, 10, 16, 17, 19]. These findings have thus validated one of the main tenets of the vasogenic edema concept, namely – the predominantly extracellular spread of edema fluid.

The other main tenet, – increase in cerebrovascular permeability, is invariably associated with alterations of the blood-brain barrier (BBB) and has been the subject of several investigations in our laboratory. The morphological site of the BBB for protein has been previously established to be the tight junctions formed by membrane fusion of adjacent endothelial cells lining the cerebral vasculature [4, 28]. Since pinocytotic

transport across the endothelium does not appear to play a major role [3] disturbances of the BBB resulting in extravascular leakage of serum proteins and spread of edema fluid must be due either to physical destruction of endothelium, creating discontinuity of the vascular lining or to opening of endothelial tight junctions. The latter is of special interest since it can be assumed that non-destructive, temporary opening of tight junctions may be operative in a variety of neuropathological conditions associated with BE.

A model for a reversible opening of tight junctions was established by application of hypertonic solutions to cerebral vasculature. It was demonstrated that various electrolytes and non-electrolytes which have little or no lipid solubility but differ in chemical and ionic properties produce a similar BBB breakdown, the intensity of which depends directly on the osmotic concentration of the solutions [27]. The effect of these hypertonic solutions was initially studied by topical application of a circular filter paper pledget soaked with a test solution to the exposed pial surface of the rabbit brain. The breakdown of the BBB was manifested by the blue staining of underlying brain tissue due to extravasation of systemically injected Evans Blue (EB) indicator. With this approach the threshold values for BBB damaging osmotic concentrations were established, as well as, the reversibility of BBB injury was determined by administering the EB indicator at different time intervals following topical application of hypertonic solutions.

In order to observe ultrastructurally the osmotic effect on the cerebral vasculature in another series of experiments, hypertonic solutions were applied to a cerebral hemisphere of the rabbit by unilateral internal carotid artery perfusion. Similarly, the osmotic concentration thresholds and reversibility of BBB injury were established. Ultrastructural behaviour of the BBB was assessed using horseradish peroxidase as the EM tracer. The EM observations revealed unequivocal pictures of the tracer passing *between* the tight junctions without evidence of cellular damage to endothelial cells themselves. Especially, the occasionally observed pictures of the tracer seemingly entrapped between two points of junctional fusion strongly imply an intravital existence of a patent for proteins route between the adjacent endothelial cells since it appears inconceivable that such pictures could be produced by lateral spreading of the tracer from some distant point of cellular disruption of the endothelial lining.

Beside the described above reversible BBB injury due to osmotic effect, there are, undoubtedly, other mechanisms for the opening of interendothelial tight junctions. Endothelial contraction induced by histamin-type mediators has been demonstrated by Majno *et al.* [21]. An interesting alteration of the BBB, which potentially could be of great significance for the problem of BE, has been observed with regard to serotonin. A traumatic serotonin release into the cerebro-spinal fluid was originally demonstrated by Sachs [30]. Later, Misra *et al.* [22] reported serotonin in CSF following acute cerebrovascular accidents and quadriplegia. Most recently, Osterholm *et al.* [26] have shown serotonin elevation in CSF, as well as in various parts of the brain after experimental head trauma. The production of BE itself by intracerebral injection of serotonin was first described by Bulle [5]. This finding was confirmed by Osterholm *et al.* [26] who reported that minute amounts of serotonin induced a marked edema of the injected cat hemisphere. Concerning the mode of action it has been shown in tissues other than brain that serotonin 1) acts on endothelial cellular cement loosening

up intercellular junctions [20] and 2) it produces a constriction of the venules [8]. The mechanism of serotonin action on cerebrovascular permeability is being currently investigated in our laboratory by Westergaard and Brightman (unpublished). In this study, since serotonin does not penetrate the BBB, is has been introduced into the brain parenchyma by intraventricular perfusion, whereas horseradish peroxidase tracer has been injected systemically.

The preliminary EM observations indicate that in regions of the brain penetrated by serotonin from ventricular lumen the capillary permeability to peroxidase remains unaffected; on the other hand, there is s striking presence of peroxidase showing invasion of vascular walls of blood vessels larger than capillaries. Thus it can be concluded that in the brain serotonin does not act on endothelial tight junctions themselves but when it reaches from outside a blood vessel endowed with cellular elements more than endothelium it conceivably produces a contraction of these elements resulting in a secondary opening of endothelial tight junctions allowing a penetration of proteins such as peroxidase tracer. This observation alone, on ability of serotonin to affect the permeability of cerebral blood vessels, should justify more extensive studies on other biogenic amines with regard to their potential role in dynamics of vasogenic BE.

An interesting behaviour of cerebrovascular permeability has been observed in cerebral ischemia. In experiments by Olsson and Hossmann [24], after a period of ischemia produced by clamping major arterial supply, an application of strongly damaging BBB agents failed to produce an increased vascular permeability. It almost appears that ischemia leads to some physicochemical alterations resulting in a "tighter" adherence of the interendothelial tight junctions. Such postischemic resistance of the BBB may persist for several days. Eventually, the ischemic necrosis of the tissue disrupts physically the continuity of the blood vessels and the resulting leakage persists until there is reconstitution of new vascular channels with normally functioning BBB. This invariably occurs after an elapse of approximately 3 weeks irrespective of the size of the ischemic infarct [25].

In general, the observations presented above emphasize the *diversity of mechanisms* which may be operative in inducing increased cerebrovascular permeability – a crucial pathogenic event in vasogenic BE.

After penetration of the endothelial barrier the serum contents are free to migrate through the extracellular spaces. Their spreading, however, shows a striking predilection for the white matter, regardless of the etiology of primary vascular damage. How much structural features of the CNS may account for this predilection can only be speculated. It is conceivable that an advancing front of vasogenic edema encounters less resistance by migrating through extracellular cannels between rather straight and orderly arranged nerve fiber tracts than by pushing through a jungle of tangled cellular structures of the grey matter.

As the area of actual vascular damage and increased permeability in vasogenic edema is usually limited and progression of edema occurs by extravascular migration through extracellular spaces [15], an ultimate extent of edema territory is largely influenced by two factors: 1) the level of systolic blood pressure (SBP) and 2) the duration of vascular injury and opening of the BBB. Simplistically, this resembles a situation with a broken main where the size of the resulting flood will depend mostly on:

a) the *pressure* in the pipe system and b) the *time* the flooding remains unchecked.

The effect of SBP on the extent of vasogenic edema has been clearly demonstrated [15]. Thus, elevating SBP about 100 mm of mercury from initial levels in the experimental cold lesion model results in edema reaching its maximal 24 hours peak within a few hours. Conversely, lowering the SBP produces a dramatic inhibition of edema development. The latter should be kept well in mind when evaluating various therapeutic measures. For example, it is still uncertain to what extent the seemingly beneficial effect of hypothermia can be attributed to the lowering of the tissue metabolic rate or to the lowering of the SPB. Also, in recent investigations on controlling edema by administration of substances interfering with catecholamines synthesis their pharmacological effect could be confused with the effect of severe hypotension which some of these compounds produce.

The duration of leakage from the injured blood vessels is of unquestionable relevance for the ultimate extent of vasogenic edema. Several situations in this regard might be pertinent. A brief, reversible disturbance of the BBB, which otherwise can be conspicuously demonstrated with dye indicators, will not allow escape of serum contents in amounts to term the involved brain tissue edematous. Such transitional BBB disturbances we have encountered, in addition to the osmotic injury mentioned, mostly in experimental conditions in which a vascular spasm appeared to be significantly involved. A reversible BBB opening without appreciable edema was observed in acute elevation of SBP [12], air embolism, acute asphyxia [23], etc. When the BBB opening persists for a longer time edema invariably develops and consistently its spread shows a conspicuous predilection for the white matter, even though the primary vascular injury is frequently located within the grey matter. If the vascular damage is associated with destruction of endothelial lining, its repair and reestablishment of the BBB will depend on how massive and how completely necrotic was the lesion, as well as, on the persistence of pathogenic factors of a primary lesion. For example, in our experimental cold lesion model the abnormal vascular leakage within the necrotized upper layers of the cortex persists for about two days, and restoration of the normal vascular permeability in this area coincides with beginning of edema resolution [15]. The duration of increased cerebrovascular permeability in an ischemic infarct has been described above. It can be assumed that in conditions such as brain tumors, abscesses, etc. where the pathogenic factors are of persistent nature the abnormal cerebrovascular permeability may stay equally chronic.

The vasogenic type of BE is, unquestionably, clinically most important and common. As much as we presently know about the dynamics of its development, the aspects of its resolution remain obscure. The only conspicuous finding from our studies pertains to a morphological evidence of uptake of extravasated protein tracers by cells of mesodermal origin (pericytes, microglia) with suggestion of a transport towards blood vessels, subarachnoidal and ventricular spaces.

The *cytotoxic* type of BE remains much less explored and understood than the vasogenic type. As stated previously [14], the basic mechanism in cytotoxic edema is related to an effect of a pathogenic factor *directly* on cellular elements of brain parenchyma causing their swelling. Since in this case the crucial event is the intracellular shift of water, which itself passes freely through the BBB, the status of cerebrovascular permeability is of no significance here and the BBB may remain entirely intact.

However, it is now generally accepted that the BBB phenomenon involves more than impairment to the passage of substances at the tight junction level and that BBB function includes also complex mechanisms for homeostatic regulation of chemical environment of brain parenchyma, among them active and facilitated transport systems. The latter could be easily affected in conditions where a cytoxic agent, in addition to inducing intracellular fluid uptake, is also responsible for alteration of brain tissue metabolic requirements.

A typical picture of cytotoxic BE can be observed in an area of ischemic infarction, where EM observations reveal an extreme swelling of cellular elements of the parenchyma, frequently associated with rupture of cell membranes. Of special interest in this connection are the observations of Hossmann and Olsson [11] who reported a remarkable suppression of mentioned tissue changes and a recovery of neuronal function if during the period of ischemia the brain was continuously perfused with a saline solution. These findings seem to indicate that it is not the deficiency of oxygen supply *per se* but an accumulation of metabolic waste products, such as lactic acid, which may be responsible for acute hydropic and destructive alterations of the brain tissue cellular elements in ischemia. This interpretation is also supported by the fact that our own efforts, as well as, of Dr. Pappius and her coworkers (personal communication) to induce experimentally a BE in anoxic conditions with retained blood circulation were persistently unsuccessful, whereas Bakay and Bendixen [12] reported that only when hypoxia was associated with considerable hypercapnia was there evidence of increased cerebrovascular permeability to proteins and incipient BE.

Until recently the triethyl tin poisoning has been one of the most typical examples of cytotoxic BE. In this condition triethyl tin, bypassing the BBB, specifically affects the myelin sheaths inducing a splitting of myelin lamellae, intramyelinic vacuole formation and a general, striking edema of the white matter [1, 18].

Most recently our laboratory became involved in investigations on a similarly interesting condition, namely – the effect of hexachlorophene on the brain. This substance has been extensively used as a germicidal agent and also has been recommended as a broad spectrum fungicide and bactericide [7]. In one investigation reported so far concerning the effect of this substance on the brain Kimbrough and Gaines [13] found a severe edema limited to the white matter which showed an intense vacuolization of myelin. In our current studies on the effect of hexachlorophene all tested animals (rats, rabbits and monkeys) proved to be susceptible to this compound. In addition to white matter involvement (Fig. 1), edematous changes were also apparent in the basal ganglia (Fig. 2). The cerebral cortex appeared at first to be resistant, however, in the monkey we have found a conspicuous swelling of subpial unmyelinated nerve fibers. Clinically, the animals given hexachlorophene showed tremors, ataxia and paralyses, especially of the lower extremities; in monkeys it was possible to observe a definite papilloedema. There were no changes in cerebrovascular permeability to protein tracers; on the other hand glucose transport was strikingly elevated. Since with proper manipulation of the dosage the edematous hexachlorophene changes can be made chronic or reversible, the study of this model appears to be especially suitable for the elucidation of various biochemical events in hydropic alteration and for studying the ways of reducing or preventing the cytotoxic edema.

The foregoing review summarizes briefly some progress in understanding basic pathophysiological aspects of BE. The amount of this new data appears rather unim-

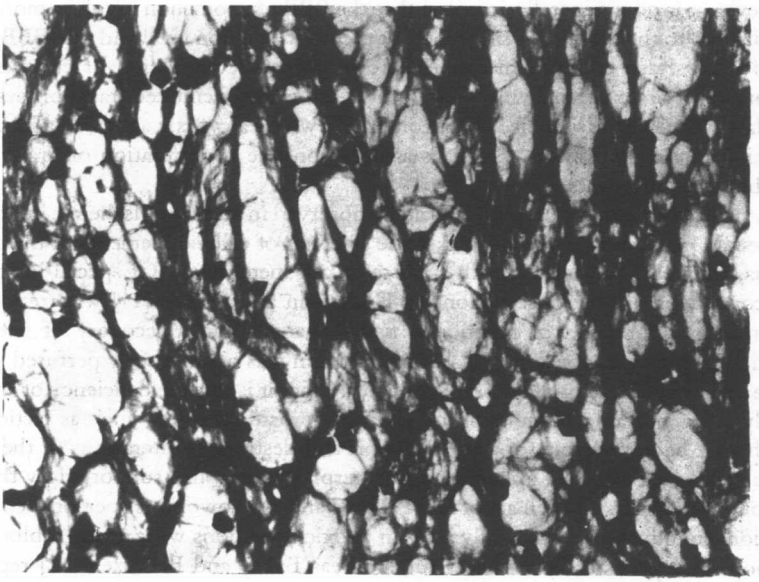

Fig. 1. Rat given 15 mgm/kg of hexachlorophene daily for 2 weeks. The extreme vacuolization of the white matter. Hematoxylin and eosin; × 260

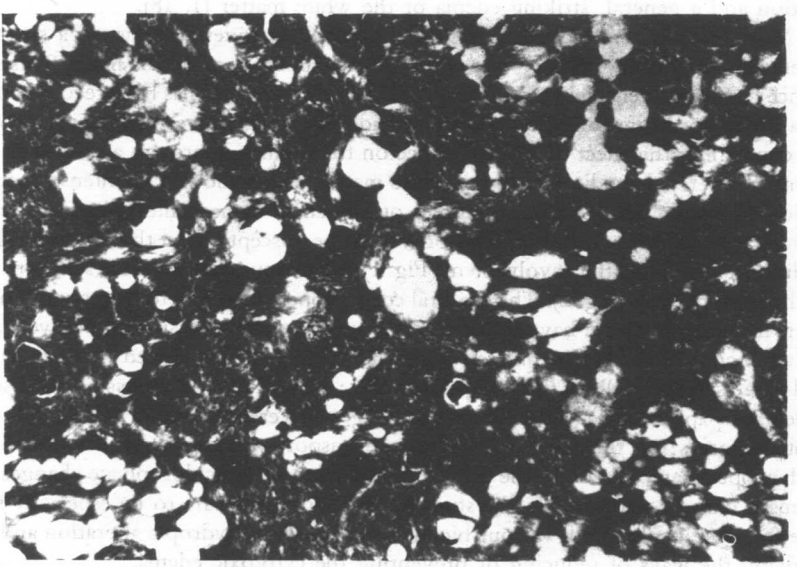

Fig. 2. Thalamic region from the same rat. Numerous vacuoles are conspicuous in the neuropil. The nerve cells appear well preserved. Hematoxylin and eosin; × 460

pressive, especially in view of undiminished urgent need for clinical management of this condition. Undoubtedly, some empirical approaches and clinical trials may bring some spectacularly beneficial results. Nonetheless, our efforts towards understanding the basic mechanisms must continue as this offers solid promise for eventual comprehensive control of BE.

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Functional Aspects of Abnormal Protein Passage Across the Blood-Brain Barrier

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With 3 Figures

Summary. The influence of functional disturbances of the brain to the passage of circulating protein tracers in a standardized chemical blood-brain barrier lesion was studied in cats subjected to different periods of global cerebral ischemia. Ischemia was produced by arterial clamping, and the chemical lesion by intracarotid injection of mercuric chloride. When ischemia was long enough to cause depolarization of the cell membranes, the chemical lesion failed to provoke extravasation of the protein tracers. In normal animals and in those which functionally had recovered from ischemia, the tracer passed through the endothelial lining of the cerebral vessels and spread into the intercellular spaces. It is concluded that abnormal passage of circulating proteins in vasogenic brain edema depends not only on the vascular lesion but also on the functional state of the brain.

Introduction

The recent finding of Rapoport *et al.* [9] on the reversible opening of tight junctions and its relation to brain edema has thrown a new light on the pathomechanism of abnormal protein passage across the blood-brain barrier (BBB). It appears from this investigation that in vasogenic brain edema macromolecules may leak between the endothelial cells of the brain capillaries which in the normal state are fused together by tight junctions [1]. This implies that tight junctions are not static contacts between endothelial cells but may be formed and released by cellular changes, e.g. cytoplasmic contractions [9].

The possibility of changes in the properties of interendothelial cell junctions has also been discussed in another experimental condition [7]. Extravasation of serum proteins caused by standardized chemical and hemodynamic lesions was inhibited by a period of transient ischemia which was severe enough to completely suppress neuronal function. The inhibition was reversible and disappeared when neuronal function returned. It is obvious that both the changes in the behaviour of the blood-brain barrier and the suppression of neuronal function were related to the metabolic disorder in ischemia. The reason for the inhibition of abnormal protein passage, however, remains unclear as long as the exact pathomechanism of protein exudation in vasogenic edema has not been solved.

* The experiments reported here were performed in the Laboratory of Neuropathology and Neuroanatomical Sciences, N. I. H., Bethesda Md., and in the Cologne laboratory.

A precondition for the study of this question is the precise morphological localization of protein tracers by which the route of exudation from the vessel lumen into the brain parenchyma can be determined. In the present investigation the protein tracers Evans blue and peroxidase were used for this purpose. Evans blue has a strong affinity to serum proteins and can be traced macroscopically and on the cellular level by fluorescence microscopy [11]. Small amounts of horseradish peroxidase are demonstrated enzymatically by the method of Graham and Karnovsky [5], and can be localized by both light and electron microscopy.

With this technique protein exudation was investigated in a standardized chemical blood-brain barrier lesion which was produced by intracarotid injection of mercuric chloride [3]. The lesions were preceeded by different periods of global cerebral ischemia and the functional impact on the cerebral cortex was controlled by neurophysiological methods.

Material and Methods

Animal preparation. The experiments were performed in adult cats. The animals were anaesthetized by a single intraperitoneal injection of 30 mg/kg sodium pentobarbital, immobilized with 10 mg/kg gallamine triethiodide and mechanically ventilated with room air. Body temperature, arterial blood pressure and end-tidal CO_2 were monitored and kept within physiological limits.

Production of Increased Cerebrovascular Permeability. Increased vascular permeability was produced by intracarotid injection of a 6×10^{-5} solution of mercuric chloride in saline according to the technique of Flodmark and Steinwall [3]. The right carotid artery was exposed and cannulated with a polyethylene tube. The solution of mercuric chloride was injected in 30 sec through the carotid catheter under a pressure high enough to expell the blood from the pial vessels. This was controlled microscopically by observing the cortical surface through a window in the skull. The volume of the injected solution depended on the blood pressure and varied between 30 and 50 ml.

Production of ischemia. The chest was opened from the left dorsolateral side and the innominate and the left subclavian arteries were exposed close to their origin at the aortic arch. Global cerebral ischemia was produced by clamping these vessels without blocking the venous outflow from the brain. In some animals the resulting increase in arterial blood pressure was counterbalanced by infusion of a ganglion blocking agent (camphor sulfonate). At the end of the ischemic period the clamps were removed and a vasoactive agent (Novadral or norepinephrine) was infused to prevent postischemic hypotension.

Neurophysiological methods. The functional impact of ischemia was assessed by recording the electrocorticogram and the response in the pyramidal tract following an electrical stimulation of the motor cortex, as has been described elsewhere [7]. In some animals cortical impedance was assessed by a four electrodes technique [6]. The electrodes were inserted in a row 1.5 mm under the surface of the right suprasylvian gyrus. A constant current pulse was passed through the outer electrodes and impedance was calculated from the voltage drop across the inner electrodes.