CEREBRAL EDEMA: CONCEPTS, PRODUCTION AND CONTROL, USING AN EXPERIMENTAL MODEL

by

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A THESIS
Presented to the Department of Pharmacology
and the Graduate Division of the University of Oregon Medical School
in partial fulfillment of
the requirements for the degree of
Master of Science

June 1964

APPROVED:

[redacted]

(Professor in Charge of Thesis)

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(Chairman, Graduate Council)

ACKNOWLEDGMENT

I wish to express my deep appreciation and gratitude to Dr. Norman A. David for his guidance and encouragement in the course of this investigation. The time spent under his supervision has been both enjoyable and rewarding, and his inspiration has made a lasting impression on me. Special acknowledgment is extended to Dr. Philip D. Gordy who spent countless hours explaining to me the wonders of the nervous system, a subject which he has mastered; also to Dr. Chen Tsai, Dr. George Austin, and Mrs. Evelyn Burkhart, all from the department of Neurological Surgery; to Mr. Lavelle Jackson, previously with the department of Neurology, for his enlightened assistance in the various physico-chemical problems encountered; to Dr. Elton L. McCawley from the department of Pharmacology for his keen suggestions and constructive criticism.

I am also greatly indebted to my fellow graduate students and to each member in the department of Pharmacology for their help and companionship; to Mrs. Frances Kemper from the department of Medical Illustration for her careful and exacting work.

Finally, I would like to thank Mrs. Helen MacLean and Mrs. Marion Bergstrom for their sincere dedication and the many days spent in typing this manuscript.

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INTRODUCTION

Cerebral edema is the condition resulting from the abnormal accumulation of a variable amount of fluid in the brain tissues due to pathological alterations in the relationship between brain fluids and electrolytes. The occurrence of this type of edema is unique in that the bony cavern of the skull limits the extent to which the brain tissues can swell. With edema there is an absolute increase in the amount of fluid and a relative decrease in the amount of dry substance. There is also a shrinking or decrease in the difference between the volumes of the cranial cavity and the brain. This difference is often referred to as the cerebral "space" (36) which under normal conditions is maintained at a fairly constant ratio (170).

It is apparent that dynamic forces are concerned in the production of cerebral edema. One theory postulates that a change in the permeability of the "brain barrier" must take place. The term "brain barrier" is used here in its broadest meaning and includes the various changes in vascular permeability which occur (49, 62, 79).

Clinically, cerebral edema is one of the fairly uniform responses of the brain to many noxious stimuli. The initiating cause may be a cranial injury, operative trauma, a circulatory disorder, anoxia, an inflammatory lesion, or a brain abscess. Or the edema may be associated with a variety of conditions ranging from poisoning to intracranial expanding lesions (62, 72, 116, 122, 136, 143, 183, 184). Of interest, also, are the many references in the older literature associating some

forms of psychosis ("catatonia"), epilepsy and general toxemias with cerebral edema. Today, the likelihood of edema being one of the basic disturbances in these conditions is rarely considered (4, 120, 121, 156).

The disturbances in the brain accompanying edema may be either cerebral hypoxia, insufficient and inadequate nutrition of the brain tissues, or increased intracranial pressure (62). All three of these pathologic conditions are mutually interrelated and may be self-perpetuating unless homeostasis is promptly restored. In this thesis emphasis has been placed on studying the increased intracranial pressure and the various changes in the brain provoked by or resulting from this condition since better technical methods and more objective criteria were available to us for correlating these phenomena with edema formation.

The use of the term "brain swelling" has been purposely avoided in order not to bring chaos into an already confused understanding of the basic problem. Particularly is this true since its existence as a separate entity now still remains on as tenuous a ground as it did at the beginning of this century. Furthermore, a considerable number of workers in this field presently regard both "brain swelling" and cerebral edema as variations along a continuum of the same abnormal process (62, 136, 92, 163).

Since cerebral edema is a pathological condition superimposed upon previously healthy tissues, a description of the characteristics of the normal brain is presented first before considering the pathophysiologic aspects of cerebral edema.

Physiology and Physico-chemical Properties of the Normal Brain

Under normal conditions the various functions of the mammalian brain are carried on in a highly coordinated manner despite the fact that brain tissue is even less of a homogeneous structure than other organs such as the spleen or liver. Structurally, the brain may be grossly divided into gray and white matter, the former constituting 45 per cent and the latter 55 per cent of the total brain volume. Neither the gray nor white matter are uniformly homogeneous within themselves. For instance, myelinated fibers can be found in various gray areas of the cerebral cortex and thalamus while, in certain species, neurons may be located deep in the white matter (165).

Water constitutes the largest fraction of brain tissue, the amount varying with the age, size, sex and weight of the individual. The greatest change in water content occurs during the process of maturation of the central nervous system (CNS) and, especially during the "phase of rapid myelination". In the human this occurs during the first two years of life. For example, the whole brain of the newborn infant contains an average of about 88 per cent of water while at the end of the most active period of brain growth, this figure drops to 81 per cent. Finally, when full maturity is reached, the water content constitutes about 77 to 79 per cent of the total brain weight (31, 32, 33, 149). Senility and old age are also accompanied by changes in the brain-water content and this parameter increases concurrently with the loss of normal tissue. Some workers believe that these changes can be correlated with the degree of gliosis, an extreme example being that seen in the atrophic brain (4).

The structural division of the brain into gray and white substances may be considered from a physico-chemical aspect in order to account for the morphological differences. The relative absence of myelinated fibers in the gray matter coupled with their presence in vast numbers in the white substance suggests a difference in water content. This is exactly what may be found experimentally. Various workers have reported comparable values of from 80 to 82 per cent water content for gray matter and 70 per cent for white matter (112, 113, 139, 150, 156). This difference in myelination is evident during the phase of rapid central nervous system growth since studies have shown that the maturation of the axon in white matter is accompanied by a greater water content diminution than that found in the maturing gray substance (32).

Another facet of brain growth intimately associated with brain-water content is the relationship of brain bulk to skull volume. The total volume of brain substance bears a consistent relationship to the cranial capacity and must be ultimately fixed by the rigid confines of the bony skull. The formulation of the Monro-Kellie doctrine (76, 101), explained later, was based on this concept and attempted to explain the derangement of brain bulk occurring in cerebral edema from a dynamic standpoint.

Under normal conditions the volume of the brain has been found to be around 10 per cent smaller than the surrounding cranial cavity (36, 120, 184). White and his associates (183, 184) have expressed this measurement as an index which they termed the "differential index of

brain volume"* and which, under physiologic conditions, was found to be about 11 per cent.

Just as a change in brain water content can be demonstrated with increasing age of the animal, a similar variation exists with regard to cerebral bulk. For instance, in man the difference in volumes between the cranial cavity and the brain substance was found to be about 2.5 per cent during infancy and 15 per cent in old age (130). This difference or "space" includes the meninges and cerebrospinal fluid in the subarachnoid space, the cisterns and the ventricles and is thought to be related to the production of cerebrospinal fluid in the brain, although the exact nature of its relationship is not well understood (183).

The solid matter of the brain accounts for only a small percentage of the total mass with about 18 per cent found in the gray matter and 30 per cent in the white matter. The principle constituents are proteins and lipids, the former found in greater concentration in the gray matter and representing about 50 per cent of the dry weight. The lipids predominate in the white matter where they constitute about two-thirds of the total dry weight. Not only are lipids found in greater quantities in the brain, but there is also a number of lipid-like proteins. These substances (an example of which are the protec-lipids) despite their high protein content, behave chemically like lipids (165).

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*Differential Index of Brain Volume

$$(DIBV) = \frac{\text{Capacity of skull} - \text{volume of brain}}{\text{Capacity of skull}}$$

. This index was previously used by K. Rieger (125) in 1885, M. Reichardt (120, 121) in 1905, and O. Rudolph (130) in 1914.

The lipids in the brain are very significant to the present study since most of the accepted values for brain-water content (including those reported in this thesis) have been determined by dehydration techniques. This involves the heating of the particular tissues at a certain temperature and for various periods of time. It now appears that, depending on the temperature and time of dehydration, a greater or lesser degree of breaking-up of fats takes place. Therefore, the values for the absolute brain water content would seem to depend to some extent on the method of dehydration (143, 160, 186). Lipids, particularly those containing unsaturated fatty acids are susceptible to fractionation when exposed to increasing temperatures such as those temperatures used in the so-called "classical" methods of dehydration. The lecithins, or phosphatidyl cholines, are of special interest in this respect. They differ from similar compounds found in other organs of the body in their greater content of highly unsaturated fatty acids. This means that lecithins have a greater number of unstable bonds which readily split up once a critical temperature range has been exceeded (83).

The chemical structure of the proteins found in the brain is similar to that seen in other organs of the body. They possess low amounts of albumen contrasted with high globulin content, especially of the beta-globulin fractions which contain the main lipoprotein components. These are the lipomucleoproteins, the phosphoproteins and the proteolipids; the first two accounting for the majority of protein-bound phosphorus in brain tissue (165).

A number of solutes, some of them of paramount importance in nerve function, are identifiable in the combustion products of brain tissue. They constitute about 1 to 1.5 per cent of the total fresh brain weight (18, 150). The principle inorganic substances are: Potassium, (mainly as potassium phosphate and chloride), sodium, calcium and magnesium. Potassium is found in about equal amounts in all parts of the brain (i.e., both gray and white matter) while chloride and sodium are present in larger amounts in the gray matter, which contains the greater percentage of water. Phosphorus, found to a greater extent in organic combination, is related more to the solids than to the water content. A higher concentration of phosphorus is found in the white matter when this is considered in relation to whole tissue. But when the amount of phosphorus is considered as a percentage of dry weight, relatively equal amounts of it are found in both gray and white matter (156). Similarly, calcium and magnesium, if expressed in terms of fresh tissue, are again found in greater quantities in the white matter (150).

When the chemical substances of whole brain are examined and the main ionic constituents expressed as a percentage of the total fresh tissue weight, potassium is found to have the highest value followed, respectively, by sodium, chloride, magnesium and calcium. Several studies (165) indicate that the brain chloride ion is in complete equilibrium with the serum chloride while most of the sodium and potassium do not bear this relationship.

Consideration may now be given to the physico-chemical environment of the brain which until recently had eluded observation. Improved

methods of tissue visualization using the electron microscope and more sophisticated ways to follow the fate of a substance using radioactive tracers have made possible the formulation of a scheme describing the interaction between the brain and its neighboring fluent entities. This scheme, first proposed by H. M. Gerschenfeld and his co-workers (50) suggests that the brain maintains an active and constant relationship with blood through the blood-brain barrier and with cerebrospinal fluid through the liquor-brain barrier. The cerebrospinal fluid, in turn, communicates with the blood through the blood-liquor barrier.

Cerebrospinal fluid (CSF) is of great importance to the normal physiology of the brain. Besides serving as a cushion in which the cerebral substance may "float", it is secreted at a fairly uniform rate by several intracranial structures including the choroid plexus, the ependyma and the lining of the subarachnoid space. Its production involves the enzyme carbonic anhydrase which, so far, has been localized both in the choroid plexus and in glial cells (51). Providing absorption of cerebrospinal fluid keeps pace with its production, a definite amount will be present at all times. Under homeostatic conditions, this also implies a fairly constant cerebrospinal fluid or intracranial pressure made more critical by the rigid encasing of the brain and spinal cord.

An exchange of drugs may occur between the brain and cerebrospinal fluid by either simple diffusion or some form of active transport (59, 118). However, drugs may reach the brain by other routes. It is well known that certain intravenously administered drugs will

exert a pharmacological action on the CNS before significant concentrations can be detected in the cerebrospinal fluid. Similarly, there is evidence indicating that drastic changes in the serum concentration of certain electrolytes, especially K^{+} and Ca^{++} result in little or no physiological aberration of the central nervous system. On the other hand, minor changes in the concentrations of the same electrolytes in the cerebrospinal fluid may cause profound neurological disturbances as evidenced by neuromuscular symptoms (166).

No less important to the present discussion is the subject of CNS-blood interaction, for other than the heart no organ is more completely dependent upon its blood supply for proper functioning than the brain. Here again the structural arrangement and division of the brain into white and gray matter must be taken into consideration as one finds different vascularity in these two areas. This is exemplified by the average figure of 1000 capillaries per square millimeter of cross-sectional area noted in the gray matter of the cerebral cortex compared to 300 per mm^2 in the white matter (77). From studies done on cats it has been possible to further delineate these differences in the form of vascularity ratios which may be calculated by dividing the number of capillaries per square millimeter by the same value found for the white matter. Using this criterion, the highest ratio was located in the medial geniculate body with lower values, in decreasing order, in the lateral geniculate body, the inferior colliculus and the cerebral cortex (8).

Despite the differences that exist in the overall rich blood supply of the brain, there is a high degree of selectivity in the transfer of the many blood-borne substances across the capillary membrane. Attempts to explain this phenomenon prompted earlier investigators to postulate the blood brain barrier (BBB). Presently the existence of such a barrier or some similar mechanism in the adult mammalian brain can not easily be denied in view of the many recent studies supporting this view (61, 166). Evidence of maturation of the brain barriers has been repeatedly observed in the laboratory and the findings of an increased selectivity with a decreased permeability on increasing age need no elaboration. An example of how this concept has been incorporated into present clinical thought is one of the explanations commonly given for the occurrence of kernicterus in the newborn (44, 46, 59, 166).

The anatomical identification of the brain barriers poses many problems of interpretation, some of which will tend to change the classical concepts of neurology and neurophysiology in years to come. Before considering this aspect, a brief review of certain histological characteristics of the brain is necessary.

Functional Histology

Of the three main different types of normal brain cells commonly referred to in the literature, the neurons have been under the most scrutiny in the past. In the rat the neurons constitute only about 20 per cent of the total cell population of the gray matter. The glial

cells are by far the most numerous in both structural layers of the brain and comprise almost the entire cell population of the white matter (with the exception of the myelinated nerve fibers). The endothelial cells, which are mostly concentrated in the gray substance, make up the smallest histological fraction (114, 165).

The importance of the neurons in this consideration resides in their medullated axis cylinders. On microchemical analysis the myelin sheath has been shown to contain various lipoid materials including cholesterol and phospholipids, certain cerebrosides (glycosphingosides) and some fatty acids. All this material is apparently deposited around the nerve processes in a concentric-layer fashion giving the myelin sheath a lamellar structure (21).

Normally, the neurons are closely connected with at least two types of glial cells, the astrocytes and the oligodendrocytes. The third classical type of glial cell, the microglia, appears to have a function similar to the one attributed to the reticuloendothelial system in other regions of the body; thus, the microglia has both migratory and phagocytic properties. These cells are usually found in close proximity to both capillaries and nerve cells and are the least common glial entity.

The astrocytes, of which investigators recognize two types, the fibrous and the protoplasmatic, have long been known because of their peculiar "sucker feet" located in apposition with the cerebral vessels. The oligodendroglia can also be found in apposition to cerebral vessels,

and, likewise, possess long beaded processes, yet they lack the plasmatic plates typical of the astrocytes (135).

Both astrocytes and oligodendrocytes are presently under intensive scrutiny because of their newly acquired importance with the advent of the electron microscope. This is especially true since they have been presently assigned the roles previously attributed to the mysterious "cerebral extracellular space". Actually, this extracellular space, which in past years had been assumed to form at least 30 per cent (165) of the total brain volume is now believed to amount to only 5 per cent or less (29, 50, 63, 92, 93).

DeRobertis (29), Gerschenfeld (50) and others look upon the astrocytes as being the central water-ion pool interposed between the blood, the cerebrospinal fluid and the cellular compartment proper, the latter represented by the neurons. As such they would be involved in the selection and transport of metabolites and fluid exchange between these compartments. Luse and Harris (92, 93), on the other hand, point mainly to the oligodendrocytes as being the cellular elements involved in a similar type of exchange mechanism. Torack and his associates (163, 164) avoid this controversy by claiming inability to distinguish astrocytes from oligodendrocytes in experimentally-induced cerebral edema preparations. They have used the term "clear glial cells" for those cellular structures functionally representing most of the so-called "extra-cellular space". Galambos (48) interprets these new concepts in the light of his modern theory of brain function wherein he postulates a continuous physiological interaction between glial cells

and neurons not only in the electrophysiologic but also in the more complex brain mechanisms.

Gross Anatomical Aspects of the Brain Barriers

When an attempt is made to describe grossly the anatomical sites of the brain barriers, again different interpretations and ideas are voiced as to the structure and location of these hypothetical units. In the ventricles cerebrospinal fluid is separated from the brain substance proper by the ependyma which has long been considered as a site for the liquor (CSF)-brain barrier. When the cerebrospinal fluid occupies the subarachnoid space, the pia and the thick layer of glial cells lining the brain (the "pia-glia") could very well represent another barrier site (26).

However, as previously mentioned, certain materials present in the blood stream need not pass into the cerebrospinal fluid in order to reach the brain. Some investigators deny the existence of a blood-brain barrier despite attributing special qualities to the brain capillaries, such as endothelial polarization (127), and the absence of fenestrations (98). Others, such as DeRobertis (29) and Tschirgi (166), recognizing that the pia does not provide a complete mesodermal barrier between the vessels and the ectodermal elements of the brain (98), postulate that the perivascular glial membrane is the most probable site of barrier activity. This theory evolved from an earlier concept that Hauptmann and Garner, 1932, and Hoff, 1933, postulated without the aid of modern visualization techniques. They thought that the relative chemical

impregnability of the brain was due to the perivascular pia-glia membrane. Finally, Hess (60) who recently demonstrated a PAS-positive "ground" substance between the unstained cell bodies of the gray matter, claimed that this material was extracellular in nature. Because of certain specific properties of this substance together with its relation to blood vessels, he identified this extracellular material as the possible anatomic location of the blood-brain barrier.

THE NATURE OF CEREBRAL EDEMA

Cerebral edema as a clinical entity is well recognized and general agreement exists as to the signs and symptoms characterizing this condition. Discrepancies arise, however, when attempts are made to explain the nature of the underlying pathologic mechanisms concerned. The existing disagreements as to what are the detectable pathologic changes taking place and the relative importance of each emphasize the relative inadequacy or lack of standardization of the methods commonly used for studying this problem.

Whether experimental or clinical cerebral edema begins in a specific area and remains localized or is generalized throughout the whole brain, an absolute increase in the amount of tissue fluid together with a relative decrease in the amount of dry substance has always been found (4, 36, 62, 73, 95, 142, 148, 149, 156, 183, 184). The increase in tissue fluid is usually of the order of only 1 to 3 per cent of the total fresh brain weight even in those cases where massive infusion of hypotonic solution was used as the experimental tool.

However, this fluid change represents a brain volume increase of around 10 per cent when calculated on the basis of the average normal brain dry weight. Since, as previously stated, there is only a 10 per cent difference in volumes between the cranial cavity and the brain substance, this accumulation of fluid noted is highly significant (36).

Brain edema, from its classical description, would appear to be essentially a disorder of the white matter (131). More recent studies, however, also include the gray matter — in some cases preferentially and in others, in combination with a more generalized process (37, 50, 62, 92, 93, 144, 154). It is also known that cerebral edema does not occur in the immature or the very young as demonstrated experimentally by Spector (149) and Seller (142). This does not, however, prevent the occurrence of increased intracranial pressure in the infant from other causes such as either over-production or under-absorption of cerebrospinal fluid. An interesting study of this particular syndrome secondary to Vitamin A deficiency was reported by Bass (9).

Evaluation of electrolytic changes in cerebral edema has been hindered to a certain extent by the almost universal practice among investigators to use their "own" units when reporting their values. Even in a single study an author might use different units to indicate values for the same electrolytes (156). The problem arises when trying to compare data from different investigators since, for example, an experimental ion might seem increased from the normal values when expressed in milliequivalents per kilogram of dry tissue. When the same figures are given in mEq/kg of fresh tissue, the same ion concentration

might now be lower than the control values because artificial dilution has been allowed to take place. (In changing units expressed in terms of dry weight to those in terms of fresh tissue weight, the relative per cent of the dry weight from the total fresh tissue weight must be taken into consideration. Thus, if in normality the dehydrated tissue is 21 per cent of the total fresh weight, in edema this would be lower, say 18.5 per cent. Therefore, one would be diluting the total amount of the specific ion for the edematous brain more than for the control were one to express it in mEq/kg of fresh tissue weight.)

In edema most authors have noticed an increased brain tissue content of sodium (Na^+); no change, a decrease, or an increase in potassium (K^+); an increase in chloride (Cl^-); either a slight increase or no change in calcium (Ca^{++}); no change in magnesium (Mg^{++}); and, no change or a slight decrease in phosphorus (29, 35, 73, 95, 119, 154, 156). In most cases the degree of electrolyte variation was related to the method used in producing cerebral edema, just as the experimental procedures varied in their degree of effectiveness.

The electrolyte values reported by two investigators merit some comment, since they deviate from other people's work including the writer's. Eichelberger et al (35) measured several electrolytes in the brains of dogs which had received "pure concussion, uncomplicated by damage to blood vessels". In the absence of "swelling or edema" (concurrent with no significant change in water content) they reported a decrease in sodium concentration 24 hours after trauma. Similarly, following experimentally induced cerebral edema secondary to water

intoxication and brain compression, Stern (154) reported a drop in sodium concentration in the presence of a significant increase in brain water content.

Inspection of the edema fluid has at times resulted in postulates which attempted to predict its nature. For instance, Stewart-Wallace (156) proposed that the edema fluid was a colloid-free "serum ultrafiltrate". (For analysis he had used mostly cases of localized cerebral edema associated with cerebral tumors.) Others have made similar claims more recently (95, 144). However, it is evident that depending on the type and/or degree of edema one might find a fluid either free, low or high in protein. The certainty of this statement has been documented both in experimental as well as in clinical material (62, 144). Presumably for this reason, Smith and associates (144) have gone as far as coining the terms "exudative" edema where the fluid was protein-containing as, for example, in lead encephalopathy, and "transudative" where the fluid was free of protein or any other macromolecular material as in the experimental type of edema seen secondary to triethyl tin administration.

Morphologically the cerebral changes associated with edema will depend on the duration, extent, and distribution of the condition. If edema is secondary to a local brain injury, such as can be produced experimentally by the application of cold to a particular gyrus, there may be initial cortical blanching, followed by intense hyperemia. Within six hours widening of the white matter underlying the damaged gyrus would be evident. The swelling of the white matter might slowly

progress toward adjacent gyri but begins to recede 24 hours following the time of injury (79).

If the edema is generalized, flattening and widening of the gyri together with a narrowing of the sulci will be evident. This can be easily predicted if one visualizes an increased intracranial pressure forcing the cortex against the overlying calvarium. Likewise, curved grooves in the posterior aspects of the cerebral hemispheres will reflect pressure of the swollen brain against the rigid bony and dural tentorium (62, 86, 122).

In animals whose brains are lissencephalic (totally devoid of gyri as in bats and rodents), the edema causes plastering of the brain substance against the calvarium. This fact may partially explain its mushy consistency and also the relative difficulty in manually separating it from its bony encasing (95). Ventrally, a crowding of the cerebral hemispheres against the optic chiasm, tuber cinereum and mammillary body results in partial displacement and in impingement of the internal carotid and posterior communicating arteries.

Increased intracranial pressure results from the shrinkage of the physiologic "space" previously mentioned. The fact that this "space" is about 10 per cent of the total cranial capacity and is limited by the rigid confines of the skull is obviously quite significant. In the presence of increased intracranial pressure, the appearance of Cushing's classical cardiorespiratory changes signifies that an acute dynamic distortion of the pontomedullary (axial) portion of the brain stem is taking place (162). (The cardiorespiratory changes associated with Cushing's investigations on increased intracranial pressure consist

of slowing of the pulse, increase in pulse pressure, elevation of blood pressure and slowing of respiration with terminal respiratory arrest and final cardiovascular failure. They have been recently attributed to an aberration in the conductivity of the pontomedullary centers for respiration and cardiovascular activity.)

Other clinical features will depend on the etiological cause of edema, manner of onset (i.e., whether acute or insidious over an extended period of time) and other similar circumstances. For instance, if cerebral edema occurs secondarily to operative trauma, onset of symptoms will most likely be 6 to 24 hours after the operation. The signs probably will be focal in nature, and consist of motor or sensory disturbances, aphasia, Jacksonian seizures and double vision. If it is related to lead intoxication, along with a history of heavy metal ingestion, one might observe as early signs anorexia and hyperirritability associated with the patient's disinterest in his surroundings. This might be followed by the fairly rapid onset of headache which, in severe cases, will be accompanied by ataxia, nausea, vomiting, drowsiness, papilledema, weakness of one or more of the extraocular muscles and usually one-sided convulsions. Objectively, one will be able to detect a substantial rise in cerebrospinal fluid pressure (65, 122, 144). In general, disorders of consciousness rank among the most regular signs of cerebral edema from any cause. More rarely, psychotic or delirious states may be encountered (62).

The histologic features usually accompanying cerebral edema can be divided into those which have been identified by means of ordinary

light microscopy and those resulting from electron microscopy studies. Thus far, however, descriptions and interpretations presented when these two techniques are compared might be open to discussion, especially since the leading authorities in neuropathology are not yet agreed upon the histological nature of cerebral edema. Nor are the obvious technical difficulties surrounding this whole problem near solution.

Starting with the premise that different etiologic agents used experimentally vary not only qualitatively but also quantitatively as to the type of edema caused may help to explain some of the existing discrepancies concerning the nature of cerebral edema. For example, in the edema secondary to both cold injury and trialkyl tin intoxication, a general loosening of the cerebral structure, interpreted by some (95) as the formation of "interstitial" spaces and seen mostly in the white matter, has been repeatedly reported. There was also a variable correlation between the amount of PAS*-positive staining dispersed throughout white matter and cortex and the severity of the edema. Swelling was present in both perivascular spaces and in glial cell bodies (163, 164).

Reid (122) described histological changes associated with the edema secondary to distilled water infusion and that associated with intracranial expanding lesions. In cases of water infusion he noted an increasing severity of the pathology if the calvarium was also removed. Thus, neurons were greatly swollen to the point of subsequent rupture in the more serious cases. The myelin sheaths also shared this feature, although in many instances the swelling was patchy. Bulbous enlargement

*PAS - Periodic Acid-Schiff

along the course of the sheath was common in the more edematous brains. The oligodendrocytes, in contradistinction to the astrocytes, showed various degrees of swelling. In the early stages their delicate processes became fragmented, shortened and soon disappeared. As the intracellular fluid increased, the nuclei also increased in size. The cells then became progressively more swollen until eventually they ruptured and became completely disintegrated.

In edema related to tumors, Reid found an almost identical picture with the exception that the astrocytes were also heavily involved, showing "elasmatodendrosis" (an alteration in size and shape), a thickening, twisting, and finally fragmentation of their processes, and occasionally changes which would result in the so-called Nissl's plump cells (i.e., large, plump, thin-walled astrocytes with short, delicate, irregular processes and no fibers).

Klatzo (79) and associates, on the other hand, in edema secondary to cold injury could show no obvious morphological changes which could be attributed to the oligodendrocytes, in contradistinction to the astrocytes. Their view was apparently shared by Russell (131).

The controversy as to the type of glial cell principally associated with edema has already been outlined. It is especially in the electron microscopic studies that these views tend to become more divergent. One school of thought led by DeRobertis (29) and his associates describes changes primarily seen in the astrocytes. Basing their assumptions on the considerable amount of experimental work done by Zadunaisky et al (191), Gerschenfeld et al (50), and others, DeRobertis describes

swelling of the astroglial cytoplasm with a decrease in electron density. The swelling comprises not only the cell bodies but also their long processes. Together with these disturbances they detect swelling of the endoplasmic reticulum with varying degrees of alteration of the mitochondria.

Another school of thought mainly popularized by Luse and associates (92, 93) points out that in edema the oligodendroglia are most prominent. They also have described the mitochondria and endoplasmic reticulum of these cells as appearing scant and widely scattered because of the increased cytoplasmatic volume. The oligodendroglial processes are also larger than usual and indent axons or astrocytic processes. Furthermore, it is their contention that the spider web-like strands which have been seen by light microscopy perivascularly in edematous brains represent distended oligodendroglial processes compressing the interspersed astrocytes "until only narrow tenuous strands remained".

Last but not least is the group of investigators among whom Torack (163, 164), Katzman (73), Evans (37) and their associates are found. They somehow have managed to maintain a middle-of-the-road attitude with reference to the neuroglial cell involved. Some term the swollen cell "clear glial cells", while others use the general term glial cells and make no attempt at further differentiation. Still others (73) describe "glial" changes in addition to massive loculations of fluid contained within myelin sheaths.

Despite the division of ideas just described, most investigators now believe that in edema the primary change (insofar as the fluid

surcharge is concerned) involves the neuroglia and only to a very small degree, if at all, the minute extracellular space.

STATEMENT OF THE PROBLEM

From the previous introductory remarks and the discussion of the nature of cerebral edema it is evident that there are still many facets of that subject which need to be understood more clearly, and their significance examined on the basis of our more recently-acquired views on the features of normal brain. It is also clear that more insight into the physiological function of the brain could be acquired by observation of selective aberrations in one or a number of controlled cerebral mechanisms.

Originally, the primary aim of this investigator was to devise a method by which cerebral edema could be produced consistently and reliably and which could be used to evaluate in a standardized fashion the effectiveness of various agents and therapeutic measures intended to lessen it. In the process of searching for appropriate methods it became obvious that no one method was universally approved by the leading authors in this field, nor was there a single criterion for determining cerebral edema which had met with their general satisfaction.

Thus, after reviewing the extensive literature and having studied the many possible indices, another objective was added to this project's original primary aim: to establish the long-argued validity of water content determinations as an index of both normality and edema. We were certain that if one method and at least one index were sufficiently

standardized so that they could be easily duplicated, we would have gone a long way toward understanding the basic problem.

Since water flow across membranes is intimately related to electrolytic changes and especially in view of the postulates by De-Robertis and associates (29, 50, 191) on the existence of a "blood brain barrier (BBB)" mechanism actively "pumping" ions so as to preserve internal homeostasis, we also decided to follow the relative variations of certain electrolytes. It was possible that a definite correlation with tissue water content could be established, at least under experimental conditions.

The stage was now set for the observation and analysis of both the histological material which had been kept under physiologic conditions and the one in which pathology had been induced. Regular light microscopy studies were, in the case of this inexperienced observer, prerequisite to the subsequent examination of tissues under the electron microscope. In this part of the project it was our aim to relate the work of such people as Pope (114) and Giacobini (51)* to any changes we could perceive in the neuroglial elements. By such measures maybe we would give more evidence to support the view that glial elements had an

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*Pope measured dipeptidase activity in various layers of the rat cortex and white matter and showed that while previously this proteolytic enzyme had been thought to be chiefly localized to the nerve cell bodies, there was now enough evidence to also implicate the neuroglia as an important site of it.

Giacobini was able to demonstrate that the enzyme Carbonic Anhydrase (CA) when dealing with the CNS, was concentrated in both glial and choroid cells. This he interpreted as supporting the idea which implicated glial cells and glial CA in a mechanism for the active transport of some ions from the capillaries into the CSF and the cerebral water-ion compartment.

important part in an active transport mechanism across the perivascular glial membrane and perhaps also across the blood-brain barrier. Also, we possibly would be able to detect any changes in the histology of the perivascular glial membrane, the pia-glia membrane or the capillary basement membrane if these were present under experimental conditions.

Finally, it should be noted that this global approach to the problem of cerebral edema and related entities has so far not been reported in the literature despite the fact that investigation of its various aspects has proceeded for a number of years at a vigorous pace.

HISTORY

A brief review of the historical highlights in the development of our knowledge of cerebral edema is offered in order to present some of the salient trends and concepts found in the extensive literature on this subject. Monro (104) in 1783 and Kellie (76) in 1824 were probably the first to propose the concept that increased intracranial pressure was directly associated with alterations in brain bulk. They reasoned that because the brain and spinal cord are incompressible and enclosed within their respective rigid encasings there could be no increase in volume of brain tissue, blood or cerebrospinal fluid without a corresponding reduction in volume of one or both of the other components. Thus, when the brain swelled there had to be either an increase in the cerebrospinal fluid pressure, a reduction in the volume of the fluid, or a decrease in the intracranial blood content.

Burrows (17) in 1843 questioned the absolute accuracy of the Monro-Kellie doctrine. While he admitted that the whole contents of the cranium (the brain, the blood and "extravascular serum") "must be at all times nearly a constant quantity", he emphasized the importance of the cerebrospinal fluid primarily as the means of replacing the loss of blood during hemorrhage. Incidentally, he believed that the amount of intracranial blood was obviously diminished by systemic bleeding.

It was not until late in the 1800's and the early 1900's that specific relationships between "cerebral swelling" (Hirnschwellung), "cerebral edema" (Hirnödem) and intracranial pressure were made. First, Rieger (125) in 1885 and later, Reichardt (120, 121) in 1905 measured and established correlations between brain volume and skull capacity and postulated that increased intracranial pressure implied an abnormal relationship between these two very important dimensions. Reichardt felt that whenever the difference between brain volume and skull capacity was less than 8 per cent, the brain could be considered swollen. He arrived at this figure by observing the differential ratio of brain volume to cranial capacity (DIBV), a method which had been previously used by Rieger (125) and again by Rudolph (130) in 1914, White et al (183, 184) in 1942, and others (4).

According to Reichardt "brain swelling" was a definite increase in the volume of the brain not due to hyperemia or to excess of free fluid. He further thought that it resulted from an alteration in the colloidal chemistry of the brain which could not be correlated with any histological changes. His observations were based on brain swelling

secondary to catatonia, epilepsy, and various infectious diseases and intoxications.

The use of the term "cerebral edema" probably preceded the concept of cerebral swelling, and its occurrence had been reported in cases of cerebrovascular accidents secondary to cardiovascular-renal disease, certain serious intoxications such as diphtheria and peritonitis, and in status epilepticus. It was generally defined at the time as an increase in brain volume secondary to a pathological accumulation of fluid in the perivascular and the pericellular spaces (136).

In 1908 Donaldson (31, 32, 33) began reporting his observations started 13 years earlier, on the growth of the rat brain and spinal cord. He placed special emphasis on the relationship between the brain and spinal cord, the water content and other variables, such as age, weight and sex. He then compared these values with those obtained for human brain tissue and showed the amazing similarity present. Some of Donaldson's values were considered previously and will be reviewed further in another section. It is interesting to note that in calculating the brain-water content, he used a dehydration technique which included weighing the tissues before and after drying. He is credited as the first to describe such a method.

In 1914 Dixon and Halliburton (30) again questioned the validity of the Monro-Kellie doctrine. After an extensive study of the cerebrospinal fluid they came to the conclusion "that the cranial contents can no longer be regarded as a fixed quantity without the power of expanding or contracting in volume". In the same year, Wohlwill (188, 54) disproved Reichardt's (120, 121) original assumptions as to the histological

nature of brain swelling by providing definite evidence of microscopic aberrations in this disorder such as the presence of Alzheimer's ameboid glial cells (i.e., astrocytes with ameba-like shapes). His observations were made, also, in cases of brain swelling secondary to toxic and infectious diseases.

Another phase in the long history of the nature of cerebral edema started with the publications of Weed and McKibben (176, 177) in 1919. They undertook to experimentally alter brain volume in cats by intravenous injections of hypotonic and hypertonic solutions. They found no lasting change in the cerebrospinal fluid pressure following intravenous injections of Ringer's solution; a marked and sustained rise in pressure after intravenous infusion of distilled water; and, an initial rise followed immediately by a marked fall in the pressure following injections of hypertonic solutions of sodium chloride, sodium bicarbonate, sodium sulfate or glucose. Their data were further substantiated by the observation of actual swelling or shrinkage of the brain on direct inspection.

The great controversy involving the relative importance of a specific type of neuroglial cell in cerebral edema began in 1926 with Penfield and Cone's (110) report entitled "Acute Swelling of Oligodendroglia". They induced edema in animals by the intravenous injection of distilled water. Their sections were first mounted in glycerine jelly in order to avoid dehydration or any related phenomena. Then, by staining these sections using a modified method of the del Rio-Hortega silver carbonate technique, histological aberrations were shown in the oligodendrocytes. These aberrations were mild in the case of the brain

sections removed one and one-half hours after injection and consisted primarily of hypertrophy of the cytoplasm and pyknosis of the nucleus. The pathological oligodendrocytes also seemed to appear here and there in patches throughout the brain. When sections were obtained 24 hours after water infusion, the brain showed scattered areas where the oligodendrocytes were vacuolated, in addition to the other changes previously described. Anatomically, the brains of the animals were grossly swollen causing the dura to be quite tense. Penfield and Cone's histological description resembled some of the changes that Reid would call attention to 17 years later in brains of animals which also had received distilled water intravenously.

Later, in 1929 Spatz (147) wrote on the differences between brain swelling and brain edema. He maintained, as Reichardt (120, 121) had done years earlier, that in brain swelling "there was a general increase in size of the whole hemisphere with an increased consistency and a dry condition of the brain substance". In brain edema, on the other hand, the brain was "softer and wetter than normal". He described "symptomatic brain swelling" in cases of cerebral tumor which affected not only the whole of the hemisphere in which the tumor lay, but often exceeded its boundaries extending into the opposite hemisphere, the medulla, the uvula and the tonsils of the cerebellum. Histologically, Spatz found the pathology limited to the white matter with the astrocytes showing clasmatodendrosis, swelling of their processes and even Alzheimer's ameboid changes. In addition he described some swelling of the neuronal processes in the affected areas.

Lastly, around 1935 Jaburek (67) proposed that what previous authors had been describing as two different entities -- cerebral edema and swelling -- were in fact different grades of pathology along the same continuum of hydration of the brain, at least insofar as it applied to cerebral tumors. By means of limited histological evidence, he proposed that during cerebral edema there was an "exudation of fluid" with the formation of spaces and holes in the tissues and separation of the neuroglial meshwork and of the perivascular and pericellular spaces. (The "perivascular spaces" described by various authors under conditions of excessive brain hydration have been termed at times the "Virchow-Robin spaces". Although these entities were originally described in normal brain tissue, there is a strong feeling today toward regarding them as being of some significance only under abnormal conditions of intracranial pressure.) Jaburek was also the first to suggest that certain areas of the brain had a special tendency to develop edema under impaired conditions and that the white matter of the hemispheres was particularly susceptible. In contrast, he believed that the gray matter of the cortex and basal ganglia, and the whole brain stem including the cerebellum and diencephalon were not particularly affected by edema.

Alexander and Looney (4) in 1938, in trying to establish meaningful relationships between cerebral pathology and certain physico-chemical brain properties, studied a number of autopsy specimens. The postmortem interval in these varied from 2 to 58 hours. They measured the water content of both gray and white matters separately by the dehydration

technique, the differential ratio of skull capacity to brain volume (DIBV) following Rieger's (125) original method, and the brain volume, the weight and the pH. Their interpretations placed most cases of cerebral edema as involving the white matter, except when the edema was secondary to occlusion of one of the larger arteries. Otherwise the gray matter of edematous brains kept the normal ratio between wet and dry weights. Also, they obtained some degree of correlation between the edema determined by dehydration techniques and that identifed by the "differential index of brain volume" (DIBV) method. Nevertheless, they thought that cerebral edema could be expressed better in terms of DIBV rather than in terms of "simple water content" determinations.

A different approach to the same old problem of abnormal brain hydration and/or increased intracranial pressure was undertaken by Williams (135) in 1939. He recorded the cortical electrical potentials of patients with an abnormally high intracranial pressure, using scalp electrodes. He also determined cerebrospinal fluid pressure by lumbar puncture. His study included 42 subjects whose cerebrospinal fluid pressures ranged from 180 to 650 mm of water and who had shown this abnormality for a period ranging anywhere from 30 minutes to seven years. Most of these patients had brain tumors and clinical complaints associated with the increased pressure. Williams was able to identify in most cases the cortical electrical tracings of the patient with the increased intracranial pressure. These tracings showed that the cortical activity was generally widespread over the cortex, extremely rhythmic

and well sustained, and had a slow frequency usually of the order of 2 per second. Although an increase in cerebrospinal fluid pressure was usually associated with some electrical abnormality, a constant relationship between the height of the cerebrospinal fluid pressure and the degree of abnormality in cortical potentials was not found in this study. Williams thus concluded that the electrical cortical potentials were more dependent upon an alteration in water balance or ionic concentration of the brain parenchyma than upon the cerebrospinal fluid pressure *per se*. He based this statement on his observations that on many occasions the intravenous injection of hypertonic solutions reduced the amount of abnormal tracings without a concomitant cerebrospinal fluid pressure change.

Another significant study was conducted by Shapiro and Jackson (143) in 1939 on autopsy material from patients who had received traumatic injuries to the head. They determined both the subarachnoid and intraventricular cerebrospinal fluid volumes and then proceeded to calculate the brain water content by dehydration techniques. They reported, contrary to the doctrine of the time which attested to a general increased quantity of cerebrospinal fluid after head trauma, that the amount of subarachnoid fluid was generally decreased and the ventricular fluid increased if traumatic internal hydrocephalus had occurred. In this latter situation part of the enlargement of the brain could be accounted for in terms of ventricular distention; thus, the brain, even though swollen, "was dry and not edematous". They also thought that part of the brain swelling could be explained by the increased blood

content from innumerable petechial hemorrhages. For the remainder of the brain swelling "the factor of unreleasable parenchymatous fluid" could be assumed only by exclusion.

The first primarily biochemical approach of note to the subject came from Stewart-Wallace (156) in 1939. He studied tissues from various areas of both gray and white matter of a series of unselected brains coming to autopsy, and also from patients who had cerebral tumors. In the latter, he used as controls the supposedly normal tissues from the opposite hemispheres. Apart from analyzing for the ions sodium, chloride, potassium and phosphorus, he also determined the tissue water content by dehydration methods. As water content for normal gray matter he gave the figure of 84.3 per cent and for normal white matter 70.7 per cent. In brains with tumors the water content for the edematous side was 83.1 per cent for gray and 81.8 per cent for white matter, contrasted to 82.6 per cent and 69.6 per cent for their normal counterparts in the opposite hemispheres. He thus concluded that the increase in fluid content (cerebral edema) was restricted to the white matter and further histological analysis led him to point to the centrum ovale as the area primarily involved. He then excluded the cortex, thalamus, the corpus callosum and the internal capsule as he was not able to identify any fluid changes in these tissues comparable to those claimed for the centrum ovale. Also, a marked elevation of sodium and chloride was found in the centrum ovale when compared to control tissues from the opposite hemisphere. The other significant change was a decrease in phosphorus on the edematous side, attributed to the "breakdown of the complex organic compounds in the cellular tissue,

associated with the degenerative processes which are seen histologically". Finally, Stewart-Wallace was unable to find any evidence in support of the concept that in association with cerebral tumors there also occurred a condition of swelling of the brain ("Hirnschwellung") which was not due to hyperemia or excess of free fluid and which could be differentiated from cerebral edema. His claim relating the edema fluid to a "serum ultrafiltrate" has been previously discussed.

Pilcher's (112, 113) studies of 1937 and 1941 deserve comment since he and his workers were attempting to duplicate cerebral trauma in the laboratory. After studying both the cerebrospinal fluid pressures and the fluid content of various cerebral areas in dogs, he stated that since no great increase in fluid content occurred after cerebral trauma, other factors such as cerebrospinal fluid volume and intracranial blood volume must be of greater importance in producing increased intracranial pressure. In a later series of experiments in which the animals' brains were exposed following trauma, he again failed to show a significant change in fluid content. Thus, Pilcher's work tended to substantiate Shapiro's and Jackson's (143) earlier claims using human material.

It then remained for White and his associates (183, 184) in 1942 and 1943 to study cerebral swelling as determined by the "differential index of brain volume" method (DIBV) in relation to cerebrospinal fluid and to blood volume in cerebral tissues. Under conditions of anoxia, such as from obstruction of the respiratory tract, failure of the respiratory center or an inadequate volume of oxygen in the breathing gas

mixture, they postulated an increase in the capillary permeability by which fluid left the blood stream and entered the adjacent tissues. When these experiments were duplicated with the new variable being experimental brain concussion, they again observed that the increase in brain volume was due to the extravasation of fluid through the capillary walls and not to the escape of red cells, to vascular congestion, or to distention of the ventricles with increased amounts of fluid.

Equally significant to the classical study of Penfield and Cone in 1926 (110) on brain swelling were the separate studies of Greenfield (54) in 1939 and Scheinker (136) in 1941 on cerebral swelling and edema associated with tumors. Greenfield gave special attention to the centrum ovale where he was able to identify constant alterations of the myelin sheaths which showed degeneration and breaking up into granules and globules. The axonal material, while less affected than the myelin, also showed some degenerative phenomena such as beading, irregularity of contour, end bulbs and cocoon-like swellings in their course. The cortical neurons appeared normal. The astrocytes, in contrast to the oligodendrocytes which appeared normal, were always swollen, appearing either as Nissl's "plump" astrocytes with an excess of neuroglial fibers or with nuclear degeneration and clasmatodendrosis. Thus, he could not differentiate between brain swelling and edema and concluded that edema was just a more severe degree of the same process as brain swelling.

Scheinker (136) studied the same type of material and described an areolar, sieve-like appearance of the nerve tissue with distention

of the pericellular and perivascular spaces. In cases of cerebral edema there was liquefaction of the nerve tissue which was probably secondary to an increased permeability of the vessel walls. In cerebral swelling he inferred that the capillaries alone were involved with little alteration of the nerve tissue proper, except for hydration and swelling of the axis-cylinders, myelin sheaths and glial cells. He also described hydration and rarefaction of the ground substance. Nevertheless, he agreed with previous authors and stated that fundamentally the two conditions were only two stages of the same pathologic process.

In 1946 Windle et al (186) were able to show significant increases in brain water content secondary to experimental cerebral concussion and secondary to water intoxication. This was in contradistinction to Pilcher's (112, 113) work a few years previously. In concussion, however, Windle could not detect an increase in the rate of cerebrospinal fluid formation or a constant increase in pressure. Nor were they able to identify in their experimental material the degenerative neuronal changes which had been typically described for the post-concussion tissue by other authors.

The previously mentioned studies of Eichelberger et al (35) in 1949 concerning brain water and electrolyte changes following cerebral concussion disagree with most of the observations of their time and those made since. Their approach, however, was an excellent attempt to study the biochemical nature of brain edema.

Elliot and associates (36) in 1949 used a method of calculating the approximate amount of brain swelling (or shrinkage) from the percentages of both water content and dry material without knowledge of the

actual brain volume. They were able then to show a definite increase in brain volume in rabbits following administration of hypotonic solutions. This swelling was made more marked whenever the brain was exposed.

Pope's (114) significant contribution of 1952 on the newly acquired importance of the neuroglia, as determined by dipeptidase activity measurements was not fully appreciated until several years later when other investigators substantiated his findings.

A study which parallels Donaldson's (31, 32, 33) monumental work on the relationship between brain water content and central nervous system growth was the one by Graves and Himwich (53) in 1955. In the rabbit they traced the change in brain fluid content from infancy through adulthood and obtained basically similar results as their predecessors.

The year 1955 also marks the beginning of an era where a widely variable number of approaches to cerebral edema would be explored. Newer techniques, better methods of histological observation and Javid's (69, 70, 71) introduction of the use of urea as a method of reducing increased intracranial pressure all provided a great incentive and renewed interest in the subject.

MATERIALS AND METHODS

Preliminary experiments were done using rabbits, cats, dogs and rats. It was finally decided however that rats would serve as satisfactory experimental animals throughout all the phases of this project. This decision was partly influenced by the fact that Donaldson's studies on the maturation of the brain and its relation to brain-water content, as well as comparisons to similar values for humans, were available (31, 32, 33). Also the uniformity of the basic cerebral mechanisms which come into play in mammalians in the presence of many noxious stimuli, coupled with their histological similarity, is well known. Finally, economic considerations obviously indicated that if a fairly sizeable animal population were to be available for purposes of statistical analysis, the rat would be suitable for such purposes.

A. Determinations of Brain Water Content.

Mention was made previously of the variability which exists in the dehydration techniques used for calculating the brain fluid content. This lack of standardization may account for some of the existing discrepancies and the inability by some investigators to detect significant brain-water changes under conditions which warrant it.

The first step in this work, therefore, was to determine the brain-water content of experimental animals following most of the procedures used by the various authors who have provided the classical set of normal values now in use (4, 31, 36, 139, 142, 148, 156).

1. "Classical" Procedure for Determination of Brain Water Content.

In this study 16 normal male Sprague-Dawley-strain rats were separated into four groups of four rats each. Each group was then assigned a number from 1 to 4, and each number in turn was divided into A or B, the letters corresponding to either half of the brain.

Rats 1A through 4B were about 13 months old (i.e., about 385-390 days old). The average weight per rat for each group was as follows:

1 (A and B) . . . . . 491½ grams

2 (A and B) . . . . . 479 grams

3 (A and B) . . . . . 467 grams

4 (A and B) . . . . . 485 grams

The rats were first allowed to become quiet by placing them in a jar containing sponges sprayed with ether so that they could be weighed accurately. After weighing, the rats were anesthetized by the intraperitoneal injection of 50 per cent urethane (1 ml per pound of body weight). Following injection, anesthesia was complete in three to four minutes. The rats' chests were then opened by a midline incision through the sternum so that the inferior vena cava and other large vessels could be severed with the animal still alive. This allowed at least part of the blood of the brain to be drained (exsanguination technique), allowing the removal of the brain to become technically easier. Next, the skull bones were teased off and the forebrains removed <u>in toto</u>, except for the olfactory bulbs which were transected. The forebrain was separated from the hindbrain by cutting through the brain stem at the level of the superior colliculi (142, 148). The forebrain was then divided into right and left halves (labelled A and B)

and each was separated from the meningeal (dura and arachnoid) coverings as much as possible and also from the larger meningeal vessels.

Each half was then placed in a 15 ml weighing bottle with lid, and then into a dessicator containing Drierite® (anhydrous $CaSO_4$ with an indicator) to prevent the specimens from absorbing any moisture from the environment. After each group of four animals had been treated in this manner, the brain halves in the bottles were weighed using a Christian-Becker chainomatic balance. This weight was termed the "wet weight". The brains were then macerated with glass rods built in the bottles. The specimen bottles were then placed with their lids off in an oven kept at temperatures ranging from 110° to 116° C.

The first drying period constituted 98 hours of continuous uninterrupted dehydration. In contrast, subsequent drying times lasted up to 405 hours and represented the result of the accumulation of shorter drying intervals.

After the tissues had been dried, the weighing bottles and their contents were allowed to cool to room temperature in the dessicators. The weight now obtained constituted the "dry weight" which, when divided by the respective fresh weight yielded the actual weights of the tissue in percentages of the original wet or fresh weight. By subtracting the last figure from 100, the percentage of weight loss was found and this was equated with the original water content of the tissue. The percentage of water in the rat brain is given after 98 hours of drying in the oven. The remaining drying periods were used to calculate the percentages of weight loss as a function of the time interval of

dehydration (Tables 1, 2, 3, 4, 5, 6 and Figures 1, 2, 3). This was done in order to demonstrate the inadequacy of the "constant weight"* method for calculating the absolute brain-water content at the temperatures and time intervals employed by the "classical" procedures. The relations between brain lipids, the temperature and time intervals of dehydration and the water content values obtained by dehydration techniques have already been described and will be further discussed when the results are presented.

2. Modification of the Classical Procedure for Brain-Water Content Determination.

Keeping in mind the perils of too high temperatures and too long intervals of dehydration, a repeat study in another 16 normal rats of nearly the same specifications as the ones used previously was attempted. The rats were again separated into four groups of four rats each, and each group was assigned a number from 5 through 8. The midbrain separation into A and B halves was similarly used.

Rats 5A through 8B were about 13 to 14 months old (i.e., about 410 days old). The average weight per rat for each group was as follows:

5 (A and B) . . . . . 367 grams

6 (A and B) . . . . . 310 grams

7 (A and B) . . . . . 436 grams

8 (A and B) . . . . . 462 grams

*By most authors' standards, two consecutive weighings within 0.00025 grams constitutes the so-called constant-weight.

In this study the oven was kept at temperatures ranging from 91° to 93° C and the tissues were weighed using a Mettler electric balance (Type H 16) which has a total capacity of 80 grams. In contrast to the previous study the tissues were not macerated this time, and the first drying period was seven hours of continuous uninterrupted dehydration. The subsequent drying times (up to 147 hours) were the result of the accumulation of variable drying intervals.

Here again the percentages of weight loss were calculated as shown before, and these were studied as a function of the time-interval of dehydration (Tables 7, 8, 9, 10, 11, 12 and Figures 4, 5, 6).

B. Brain Electrolyte Determinations

1. Acutely Treated Animals and Their Controls (TETA)

Following determination of the brain-water content values, the dehydrated brains were weighed, then ashed separately in a muffle furnace at 550° C in nickel crucibles for three hours. The ash was dissolved in 0.2 N HCl and made up to a volume of 25 cc. These solutions were then analysed by flame photometry, using the Beckman DU spectrophotometer with flame attachment and photomultiplier. The gas mixture was oxygen and hydrogen. The wave lengths employed for the cations were: Calcium, 622 mμ; sodium, 589 mμ and potassium, 766 mμ. The cation concentration of the stock solution from which the standards were prepared was sodium 150 mEq/l, potassium 5 mEq/l, and calcium 5 mEq/l (78, 160). The standards were made by diluting the stock solution in a ratio of 1:100 for both sodium and calcium and in a ratio of 20:100 for potassium. (For calculations, see Appendix.)

2. Chronically Treated Animals and Their Controls (TETA)

The dehydrated brains were ashed and treated as before and their electrolytic solutions also analyzed with a Beckman DU spectrophotometer with flame attachment and photomultiplier. The gas mixture used this time was oxygen and acetylene. The wave lengths employed for the cations were: calcium 422 mμ, sodium 589 mμ, and potassium 768 mμ. The cation concentration of the stock solution was the same as for the acutely treated animals. Similarly the dilutions were the same except for calcium, whose stock solution was diluted 1:25. (For calculations, see Appendix.)

C. Methods by which Cerebral Edema Can Be Produced.

From the time Weed and McKibben (176, 177) first produced brain volume alterations (1919) in the laboratory to the present time a considerable number of procedures for the experimental production of cerebral edema have been designed. Most of them have, in some way, tried to duplicate some aspect of the pathological condition observed in the human. Since the list of methods used throughout the literature is quite extensive, most of them will just be mentioned together with their appropriate references and only occasionally will detailed descriptions be given.

The description of the method used in this study, together with a brief historical note, will be followed by a list of most other procedures.

I. The Experimental Production of Cerebral Edema by the Use of the Triethyl Tin Compounds.

Of all the agents used in the experimental production of brain edema, few seem to be as reliable and as consistent in their effect as the alkyl tin compounds, especially the triethyl tin derivatives. The reason for their relative obscurity lies in the fact that most of the tin compounds are poorly absorbed from the gastrointestinal tract due to their insolubility in body fluids. Thus, when tin was fed to rats by adding it to their drinking water in the amount of 2 mg/day, it was found that from 98 to 99 per cent was excreted in the feces (43). Tin itself, when taken per os, is practically innocuous. However, inhalation of tin fumes can result in a benign pneumoconiosis. Its inorganic salts are also supposed to have very little, if any, toxicity except for the double salt of tin with sodium tartrate. In a study conducted by Seifter and Rambousek (141) this compound when injected intravenously produced acute injuries to the central nervous system mainly, and also to the gastrointestinal system. It has been found, nevertheless, that stannous sodium tartrate is 20 times less toxic than TET (triethyl tin) on the basis of its tin content. Thus, the full toxicity of tin is observed almost exclusively from its organic compounds: the alkyl tin derivatives (16).

Before 1950 only eight articles had been published on toxicity with most of these coming from German investigators who were concerned with the possible contamination of food preserved in cans (90). At the turn of the century, the general impression was that no serious risk

attended the use of tin in food containers because of the lack of reports on its toxicity.

While it is uncertain at present whether or not tin plays an essential biological role in the body, small amounts of tin are present in practically all animal and human organs. In addition, tin is found in most soils and plants. It was calculated that a 70 kg man must contain approximately 0.352 grams of tin. The tin present in some of our modern prepared foods is mainly due to the use of tin-lined food containers (16). For instance a study dealing with cheese wrapped in tin foil discovered the presence of this compound in amounts ranging from several hundred to 2,000 ppm (23).

Renewed interest on the subject of organotin toxicity resulted from its relatively recent use in the production of fungicides, insecticides and anthelmintics for poultry. Van der Kerk and Luijten of Utrecht, The Netherlands, (90) are chiefly responsible for a considerable part of the original work on its toxicity and for suggesting its application as a fungicide. Triethyl tin compounds are also now used as stabilizers in polyvinyl plastics and in chlorinated rubber paints (167).

In 1958, 217 cases of poisoning (110 of which were fatal) were reported in France by Alajouanine et al (1) from the use of a proprietary preparation known as Stalinon^{R} in the treatment of furunculosis, staphylococcal skin infections, osteomyelitis, anthrax and acne. This drug, taken orally, contained diethyltin diiodide dissolved in linoleic acid and, apparently, also contained impurities of triethyl tin and monoethyl tin. The total amount of diethyltin diiodide apparently ingested by these people was of the order of 3.0 grams/person (16, 157).

In most of the patients poisoned by Stalinon^{R} the first symptoms began about four days after starting the drug and consisted usually of severe persistent headaches. This was followed shortly after by vomiting, vertigo, abdominal pain, photophobia, rapid loss of weight and retention of urine. In a few patients there was also a marked hyperglycemia. In the most serious cases there was either transient or permanent paralysis, psychic disturbances, papilledema, convulsions and coma. Death was usually due to respiratory or cardiac failure. In the surviving patients, residual symptoms included persistent headache, asthenia, partial paresis, areas of anesthesia, diminished visual acuity, flaccid paraplegia and incontinence. At autopsy the most significant lesion found was edema of the white matter of the brain (16, 103). Alajouanine (1) himself believed that the best criteria for detecting Stalinon^{R} toxicity was "the detection of a decreased level of consciousness and an increased tendency to sleep".

Meanwhile, concurrent work done by Stener, Barnes and Duff (157) and Magee, Stener and Barnes (95) showed that the lower alkyl tin compounds, especially triethyl tin, had a specific effect on the central nervous system by producing cerebral edema. The dialkyl compounds *per se* did not elicit this type of response from the brain but acted as severe skin irritants and caused inflammation of the bile ducts. These workers then went on to propose that the TET compounds, being about ten times as toxic to rats as the diethyl tin when given orally, had contributed up to ten per cent of the theoretical amount of diethyl tin ingested by the Stalinon^{R} victims.

The alkyl tin derivatives have been used experimentally in various ways to produce cerebral edema. Consideration will be given here only to those specific compounds which, as stated before, are the most toxic members of the series and, therefore the most likely to elicit cerebral edema. The first types of triethyl tin compounds to become available for experimental use were triethyl tin sulfate and triethyl tin hydroxide. Both substances are white powders and very insoluble in water. Both are volatile with steam and decompose upon the presence of heat or easily associate themselves with the carbon dioxide in the atmosphere. However, for use in the present study, it was possible to obtain only the acetate and chloride salts of triethyl tin. It was decided to use triethyl tin acetate (TETA) because of the readiness with which it dissolves both in water and in normal saline.

In designing experiments to produce cerebral edema, most of the procedures devised first by Magee et al (95) and later by Terack et al (163) were followed. Thus, techniques were employed to produce both an acute type of edema developed during a period of 24 to 48 hours and a chronic type elicited over a period of 14 days.

1. Acute Production of Cerebral Edema

Twenty-eight male* rats, Sprague-Dawley strain, weighing an average of 201 grams were separated into six groups of four rats each, and four groups of one rat each. Nineteen rats, matched for strain and sex, and whose average weight was 207 grams, served as

*In the Results section, values for only 27 rats are given since one of the single-rat groups died overnight, and was only found after a considerable degree of cerebral autolysis had taken place. Thus, the various brain determinations were not carried on in this animal.

controls. The control animals were also separated into four groups of four rats each and three groups of one rat each.

The experimental group was then injected intraperitoneally with a triethyl tin acetate (TETA) solution (0.2 per cent solution weight/volume in normal saline) containing 2 mg/cc. This preparation had been previously buffered to a pH around 7 since the pH of the solution originally was about 5.4. Each rat received approximately 10 mg of TETA/kg of body weight (i.e., about 2 mg/rat).

The control animals were injected with approximately similar quantities of physiological saline.

All rats were then placed back in their cages and allowed to eat and drink ad lib until sacrificed 48 hours later. At that time, the same procedure for sacrificing the animals and determining their brain-water content was used as the one mentioned previously in part A.2 of this Section. One exception, however, was that these animals had not been partly anesthetized by placing them in a jar containing ether-sprayed sponges, since the rapidity with which the operation was performed made this unnecessary. Also, the brain tissues were only dehydrated for 18 hours since this duration of time was found to be ideal for purposes of brain-water content determinations.

2. Chronic Production of Cerebral Edema

A new group of 40 male Sprague-Dawley rats of the same specifications as above, and with an average weight of 143 grams, was separated into groups of four. These constituted the experimental

population. On the tenth day 28 of the surviving animals made up the new experimental population which was composed of seven groups of rats each. The control group included 28 animals, matched for strain and sex, and with an average body weight of 148 grams per rat. The control animals were divided into one group of 14 pair-fed rats (i.e., animals allowed to eat and drink only as much as the experimental group did) and another group of 14 animals who were permitted to eat ad lib.

The experimental group received TETA in both their food (powdered Purina Chow) and their water in a concentration of 25 parts per million, weight/weight, and weight/volume, respectively. They were then allowed to eat and drink ad lib, with the consumed amounts of food and water determined every day for purposes of applying these figures to the pair-fed group. Both the ad lib group and the pair-fed control groups were also fed powdered Purina Chow placed in metal containers at the bottom of the cages.

All animals were weighed every second day and carefully observed at least three times a day in order to accurately record the onset of any symptoms.

Beginning on the tenth day and continuing through the fourteenth day, all animals were sacrificed and their brain fluid contents determined.

A second chronic TETA study was conducted later for the purpose of providing specimens for histological examination, including both light and electron microscopy. This time the population was made up of 20 experimental rats and six pair-fed animals plus five ad lib controls.

Representative brain samples were obtained in order to determine both electrolyte concentrations and water content values. The rats were of the same specifications as above and their average body weights were 146 grams for the experimentals, 145 grams for the pair-feds, and 150 grams for the ad lib control group. Body weights were also recorded so that between the first and the fourteenth days from four to six weighings had been recorded.

II. Other Experimental Methods Used for Producing Cerebral Edema

An attempt has been made here to include most of the commonly used experimental procedures for eliciting cerebral edema in vivo. Also included are a few procedures which, although not recorded in the literature as such, have been known to produce cerebral edema clinically.

Classification of Methods

These various methods for producing cerebral edema may be classified as follows:

1. Toxic
   a. "Water-Intoxication" methods.
      i) Using distilled water as the agent: The original work using this procedure was done with cats by Weed and McKibben (176) in 1919. These workers injected distilled water intravenously in amounts ranging anywhere from 20 to 100 ml per cat. At the time, their impression was that the degree of cerebral swelling following distilled water was not too dependent upon the absolute quantity of water injected. Modifications of this procedure were later undertaken by numerous

authors, among them Reid (122) in 1943, Windle et al (186) in 1946, and Seller and Spector (142) in 1963.

ii) Using other hypotonic solutions: Elliot and Jasper (36) in 1949 described cerebral edema in rabbits induced by injection of 0.1 per cent glucose solution into the femoral vein. The infusion rate was about 2 ml/min for a period of 60 to 75 minutes, so that 120 to 150 ml of fluid were administered. According to their calculations, this amount of fluid would correspond to 2.5 to 4.0 liters in a 70 kg man.

b. Chemical Agent or Drug Toxicity Methods

i) Triethyl Tin: This method has already been outlined in detail. Investigators who have recently experimented with this type of compound are: Streicher (158) in 1962 and 1963 (159), Katzman et al (73) in 1963, and Reed et al (119) in 1963.

ii) Dexedrine: Isolated hints that this compound could induce edema were finally documented when Ong (107) in 1962 reviewed the literature and reported two cases in children, one of them three years old. This patient had ingested 320 mg of the drug and died ten hours later in a state of exhaustion. Acute internal hydrocephalus and cerebral edema were found at autopsy.

iii) Sodium Cyanide: Wheatley (182) and Ward (172) experimented with various doses of sodium cyanide in both cats and monkeys. These workers found that from 1.4 to 1.6 mgm/kg of body weight of this material in the previously paralyzed (and artificially ventilated) cat produced a marked swelling of the brain, notably the diencephalon.

iv) Lead Encephalopathy: This subject needs no elaboration and it will suffice to mention that Smith et al (144) in 1960 reported 16 cases in all of which the dominant clinical features were increased intracranial pressure and acute cerebral swelling. As already pointed out they considered this type of edema as being of the "exudative" kind, that is, where the cerebral edema fluid contained protein.

2. Traumatic

a. Extreme Temperature Exposure to Localized Brain Areas

i) Thermal injury: Lee and Olszewski (85) have reported cerebral edema affecting mainly the white matter in rabbits sacrificed 16 to 24 hours following cortical "coagulation". This injury was accomplished by first raising a scalp flap, next "cleaning" the periosteum from a skull area of approximately 2 sq cm and then applying a heated brass rod against it. This rod, 0.3 cm in diameter, had been heated over a gas flame for three minutes, and was then firmly pressed against the bone for 20 seconds. This procedure was repeated four times.

A less drastic procedure which was tried by the writer was the exposure of an area of the brain (usually frontal cortex), 1 cm in diameter, to a 250 watt infrared light lamp for a few minutes at a time. Swelling could be roughly correlated with length of exposure and distance between lamp and brain area. These observations were made both in rabbits and cats.

ii) Cold injury: An ingenious method for the production of brain edema was reported by Klatzo and associates (79) in 1958. After

exposing an area of the cortex 15 mm in diameter, a steel plate (whose stem was fixed to a glass system in which a mixture of dry ice in acetone was circulated) was applied by light touch for 20 seconds. This procedure, which was performed in cats, demonstrated edema in animals which were sacrificed at 6 hours or longer following cold injury.

A modification of this method was developed by Torack and associates (163) who directly applied carbon dioxide ice to the exposed brain area of albino mice for a period of 20 to 30 seconds. Histological preparations demonstrated maximal alteration, 24 to 72 hours after ice application.

b. Trauma by Brain Concussion

Trauma as an experimental means in this problem was popularized by Pilcher (112, 113) in the late 1930's and early 1940's. By using a special set-up which maintained the head of the animal (dog) rigid, he was able to uniformly traumatize brains with varying degrees of weights - usually 500 to 1000 grams. Pilcher's method was applied to guinea pigs in a less uniform fashion by Windle et al (186) who, nevertheless, succeeded in demonstrating a significant increase in water content in the concussed brains.

c. Trauma by Stab Wounds

Foley et al (44) in studying the effects of steroids in brain edema produced swelling by inserting a blunt probe through a small bone defect in the skull for a distance of about 5 mm. The guinea pigs were then sacrificed at varying times up to 12 days postoperatively.

d. Trauma by Compression - Using Balloons

Several authors in the past have experimented with balloons as a method of inducing cerebral edema. Some have passed pediatric catheters into the cisterna magna in dogs or other large sized animals, while others have inserted deflated balloons through a defect in the skull. In both cases the bulb of the catheter or balloon was inflated using fluid. Stern (154) used this last method in cats, making a 0.5 cm opening in the skull and then inserting a collapsed condom balloon with a capacity approaching 5.0 ml. The balloon was placed extradurally and after fixation was inflated so that brain compression would exist for some 30 minutes. When this method was coupled with simultaneous water infusion, the animals developed a more severe degree of edema than they had with either method alone.

e. Trauma by Craniotomy and Simple Exposure of the Brain Substance

Here again, this phenomenon, well known to neurosurgeons, has been repeatedly used experimentally, even though the degree of pathology cannot be uniformly relied upon in all instances.

3. X-Rays and Other Ionizing Radiation

Besides reports of isolated cases in neuropathology texts such as Greenfield's (55) there is an important experimental study by Russell and associates (132) which deserves mention. They submitted rabbits to a single X-ray dose of 2,850 r. and were able to demonstrate lesions after a latent period of approximately 100 days. It was interesting to note that the delayed irradiation-necrosis lesions were accompanied by an increase in capillary permeability.

The clinical studies referred to previously (55) described a swollen cortex, overlying a poorly demarcated white matter in those exposed patients. Also, latent periods ranging from months to years in a few cases, in which patients would experience few or no symptoms, were emphasized.

4. Anoxic

a. Anoxia Induced by Various Breathing-Gas Mixtures and/or by Impairment of Respiration

White et al (183, 184) in 1942 and 1943 studied the effects of anesthetics such as sodium pentobarbital (Nembutal^{R}) and ether and suggested that these did not produce striking alterations in the permeability of the cerebral capillaries or in the cerebral volume unless they were combined with an inadequate airway or a breathing mixture containing less than 10 per cent oxygen.

Therefore, whenever anoxia occurred, whether it would be from obstruction of the respiratory tract, failure of the respiratory center, or accessory organs, or from an inadequate volume of oxygen in the inspired air, permeability of the capillaries was increased and fluid rapidly left the blood stream and entered the brain tissues.

b. Local Anoxia Induced by the Injection of Oil or Other Embolic Agents

Edstrom and Essex (34) in 1956 introduced the technique of cannulating the internal carotid artery of dogs and injecting embolizing material to produce a very consistent and rapid increase in intracranial

pressure and swelling of the brain. These changes were present in the absence of any obvious systemic hemodynamic aberration. The embolizing agents used were either peanut oil in doses of 0.01 ml/kg of body weight, or cornstarch USP in doses of 1 to 5 mg/kg body weight. The latter was given as a suspension in less than 1 ml of saline solution.

5. Anoxic - Ischemic

Spector (148, 149) is one of the recent investigators who has been studying cerebral edema by producing anoxic ischemia. Briefly, the right common carotid artery or, more often, the internal carotid of rats was ligated in the neck, while the animals were under anesthesia. After the animals had stabilized, they were rendered hypoxic by placing them in a nitrogen atmosphere until they became apneic. They were then taken out of the nitrogen jar and "revived and placed back in it once more" for a variable period of time. Using this procedure, edema was first detected four hours after the anoxic-ischemic insult, and reached a peak by 19 to 25 hours.

6. Ischemic

Levine and Klein (86) in 1960 were able to demonstrate both marked cerebral edema and increase in brain bulk, and also a vicious-circle mechanism involving infarction and swelling in those rats who had undergone bilateral common carotid ligation alone.

Their findings were again documented by Spector (148) who ligated both common carotid arteries and then proceeded to show a very significant increase in the brain water content of the survivors 24 hours afterwards.

7. Infectious

The older literature describes cases of cerebral edema or brain swelling in patients with infectious diseases more often than do the many publications since then. Nevertheless, the association is still seen often enough and, in some cases, as in acute brain abscesses, it provides us with the main localizing neurologic signs for diagnosis. In acute brain abscesses it is the edema, rather than the space-occupying lesion, which results in the more severe neurologic manifestations and the generalized increase in intracranial pressure (72). Accordingly, investigators such as Levine et al (87) and Katzman et al (74) have been inducing cerebral edema by the use of certain substances from infectious organisms. Their purpose, of course, has been to duplicate in the laboratory the brain response to inflammation. Among the substances being used are PPD (purified protein derivative of tuberculin), lipopolysaccharide from Escherichia coli O111:B4, pellets of killed and dried Staphylococcus aureus or Mycobacterium tuberculosis and polysaccharides from pneumococcal or cryptococcal capsules. All these substances were implanted in or around the white matter through an opening in the skull.

8. Allergic

It is realized that the separation between this type of experimental procedure and the one described above is more artificial than real; in both cases a strong hypersensitivity component seems to be a factor in the general brain reaction. In this group belong substances such as crystalline bovine albumin (87) which are known to induce either local or generalized allergic reactions.

9. Miscellaneous

Implantation Procedures. - Other methods using implantation techniques have already been outlined; the procedures mentioned here employ materials which are either inert substances such as graphite or organic and inorganic compounds such as copper sulfate, silver nitrate and triethyl tin (87). All of these materials were implanted into the brain in the form of pellets. It is interesting to observe that the edema these substances produce is quite similar to the edema seen in human brains as a result of abscesses or tumors, and also to the edema seen in animal brains secondary to experimental cold, thermal or other injuries.

D. Histological Techniques

The preparation of the animals for this phase of the project was described previously under the subject of chronic production of edema. Microscopic specimens from experimental, pair-fed and ad lib control animals were available for the following studies:

(1) For Light Microscopy Examination

(a) Cajal's Gold Sublimate for Astrocytes

Paraffin sections. (After Scharenberg (134) and Naumenko (105) with some modifications by the writer.)

1. Fix tissue in Bromformalin (or 10% formalin). (For a small tissue fixation about a 24-hour period of time was required; for a large tissue, up to seven days' time was needed.)

2. Bring tissues to water dehydration and imbed in paraffin block.

3. Cut sections at 10 to 20 microns. Bring to water.

4. Put into following solution (on slides and leave from one to three days):

Ammonium bromide	0.6 gram
Formaldehyde	14.00 ml
Distilled water	100.00 cc

5. Dip once in distilled water.

6. Place in mixture of 8 ml of 1% gold chloride (yellow) and 40 ml of distilled water. Then immediately add 6.4 ml of 5% mercuric chloride. Mix well by moving slides around within jar using plastic forceps.

7. Keep in dark 6 hours or longer.

8. Rinse 2 to 5 minutes in distilled water.

9. Put in 5% sodium thiosulfate for five minutes.

10. Rinse again in distilled water for 2 to 5 minutes.

11. Dehydrate and cover.

With this technique it has been found that pathological cells (astrocytes) stain better than normal.

(b) Cajal's Gold Sublimate Method for Protoplasmic and Fibrous Astrocytes (22).

Frozen sections.

For fixation use Cajal's fixative as before.

Materials: Mercuric chloride 0.4 gm
1% gold chloride (brown or yellow) 10 ml
Distilled water 60 ml

Method: Dissolve the mercuric chloride in the distilled water with the aid of gentle heat; cool and then add the gold chloride. This solution should be prepared immediately before use.

The successive steps then followed are:

(1) Fix pieces of tissue 3-5 mm in thickness in F.A.B. for 3-8 days.

(2) Cut frozen sections to a thickness of 15-20 μ.

(3) Store sections in F.A.B. until ready for staining.

(4) Rinse well in distilled water (2-3 changes).

(5) Treat in gold sublimate solution in a flat covered dish in the dark at 22° C for 4-8 hours or longer until sections are deep purple in color. Sections must lie flat without creasing or over-lapping.

(6) Rinse rapidly in distilled water.

(7) Fix in 5% sodium thiosulphate.

(8) Wash in distilled water.

(9) Float on to clean slides.

(10) Dehydrate, clear, and mount in Canada balsam or D.P.X.

Using this technique it is found that astrocytes (protoplasmic and fibrous) appear red-black. (Frozen sections of formol saline-fixed tissue may be impregnated, but they should be treated overnight in F.A.B. (above) or for one hour in 5% hydrobromic acid (supplied commercially as a 40% solution)at 37° C; then continue from Stage 4 above.

(c) Schiff's P.A.S. (Periodic Acid Leucofuchsin) Technique. (Modified from H. J. Conn and M. A. Darrow's Staining Procedures (19), 1960.

Tissues are fixed in 10% formalin and embedded in paraffin. The sections are then cut at 5 microns.

Schiff's reagent is prepared as follows:

Materials:			
	Boiling water (distilled)	425	cc
	Basic fuchsin	5	gm
	Sodium metabisulfite	9.5	gm
	1 Normal 1% HCl (add later)	75	cc

Method: Dissolve fuchsin by pouring boiling water over it (bring to boil).

Cool to 50° C. Filter.

Add HCl and sodium metabisulfite.

Allow to stand. Add charcoal to decolorize partly. Filter and store in refrigerator at 5° C.

Paraffin sections should be cut at 6-10 microns. Then go through following steps:

1. Bring sections to water. "Oxidize sections with periodic acid aqueous solution for routine demonstrations."
2. Immerse in 1% periodic acid for 5-10 minutes.
3. Wash in running tap water for 5 minutes. Rinse in distilled H_2O.
4. Stain in Schiff's reagent for 10 minutes (see below).
5. Transfer directly to first sulfite rinse for 1 minute.
6. Transfer to second sulfite rinse for 2 minutes.
7. Transfer to third sulfite rinse for 2 minutes.
8. Wash in running tap water for 10 minutes.
9. Can counterstain with light green or diluted hematoxylin.
10. Dehydrate, clear and cover.

Baths of sulfite rinse, to fill 3 coplin jars - use:

10% potassium metabisulphite 7.5 ml
N/1 Hydrochloric acid 7.5 ml
Add to 135 ml of distilled water. This solution must be fresh.

(d) Mallory's Phosphotungstic Acid Hematoxylin (PTAH) - Modified from H. J. Conn and M. A. Darrow's: Staining Procedure (19), 1960.

Tissues were fixed in either Cajal's fixative or in 10% formalin and embedded in paraffin (Sections cut at 5 microns).

Materials:

Hematoxylin	1 gm
Phosphotungstic acid	20 gm
Distilled water	1000 ml

Method: Dissolve the hematoxylin and the phosphotungstic acid in separate portions of water, the hematoxylin with the aid of gentle heat. When cool, combine. Ripening can be accomplished at once by adding 0.177 gm $KMnO_4$ (potassium permanganate).

Then go through following steps:

1. Following sectioning of slides, remove paraffin in the usual manner.
2. Tissues should next be placed for three hours in saturated aqueous $HgCl_2$ in paraffin oven at 57° C, rinsed briefly in tap water, placed five minutes in Lugol's iodine, rinsed in tap water, placed 5 minutes in 5% aqueous sodium bisulfite ($Na_2S_2O_3.5H_2O$) and rinsed again in tap water.

3. Place sections for 5-10 minutes in 0.25% aqueous potassium permanganate.
4. Wash in tap water.
5. Place for 5 minutes in 5% aqueous oxalic acid.
6. Wash well in tap water.
7. Stain overnight in the staining solution described above.
8. Wash tissues briefly in tap water; dehydrate in alcohol or acetone.
9. Clear and mount in balsam or synthetic resin.

Results: Neuroglial fibers - deep blue

Nuclei - deep blue

RBC's - deep blue

(e) Azur II - Methylene Blue

The staining solution is prepared by mixing equal parts of Azur II and methylene blue. For example, to make 50 cc:

Materials:

25 cc of 1% Azur II (in distilled water)
25 cc of 1% Methylene Blue in Borax solution.

Mix.

This technique was used to stain the "thick" sections (1 micron) of the blocks which were to be prepared for electron microscopy examination.

Then go through following steps:

1. Mount section on slide in pool of water, then let dry on hot plate set at 60-80° C.

2. Place in 1% periodic acid for 5 minutes.
3. Wash with distilled water.
4. Add a drop of stain described above.
5. Place slide in hot plate for 5 seconds.
6. Rinse off excess stain with distilled water.
7. Immerse slide in xylene, drain and mount in medium.

(2) For Electron Microscopy Examination (146)

(a) Tissues were fixed instantly after removal from animal, in 2% Osmium tetroxide buffered with verenal acetate (with sucrose). The tissues for fixation were cut so as to be 1 mm or less in their greatest diameter. After being placed in the fixative solution, tissues were refrigerated for two hours.

(b) Dehydration was accomplished by immersing tissues in 50% ethyl alcohol for 5 minutes, in 70% alcohol for 10 minutes, in 95% alcohol overnight and, finally, in 100% alcohol for 30 minutes - 3 times. Tissues were then placed in propylene oxide twice, for 30 minutes each time.

(c) Infiltration was accomplished by placing tissues in a mixture of 1/2 Epon and 1/2 propylene oxide for 2 hours.

(d) Tissue was then placed in 100% Epon before undergoing polymerization. This was accomplished by maintaining capsules (containing tissue embedded in Epon) at 37° C overnight, at 45° C all the next day, and at 60° C for all the following night.

(e) The tissues were then sectioned with a Porter-Blum microtome to a silver-gold hue (60-100 millimicrons).

(f) Mounting was accomplished using carbon-coated copper grids.

(g) Staining was done with uranyl acetate where tissues were immersed for 1 hour at room temperature. (Tissue must be side down on drop of stain.) Tissues were then rinsed in distilled water and dried on filter paper.

The examining electron microscope was an RCA Type EMU - 3F.

The staining solution (uranylacetate) was prepared as follows:

8 grams of uranyl acetate were dissolved in 100 cc of distilled water. This must be kept refrigerated and must be centrifuged before use.

E. Selection of Dehydrated Brains for Electrolyte Determination

For the acute TETA study two dehydrated brains were chosen randomly out of each 4-rat group, and three were taken out of the 1-rat group series. This meant that since there were six groups of rats each (in the acutely treated animals), and two were taken out of each group, the number of brains was 12; then three rat brains were added from the single-rat group (also from the acute series), making a grand total of 15 brains. That is, 15 brains of the 28 which had been previously examined for water content. The controls for these acutely treated animals were selected in the same fashion, the number in this case being 11.

The reason all brains from the treated series and their controls were not analyzed for their cation content was due to the time factor involved. It must be remembered that each tissue had to go through a time-consuming process, including weighing, ashing, extraction and analysis by spectrophotometer. It was then settled that "representative" tissues from each group of rats would be analyzed instead.

For the chronically treated animals and their controls, similar reasoning was used. The exact number in each case will be found in the representative charts of the Result Section.

RESULTS

This section will review most of the meaningful data obtained in this study. Some of the material not included relates to water content values for gray and white matter and other brain areas in dogs, cats and rabbits. These observations were obtained in both normal and edematous brains, the edema having been induced by a variety of techniques including anoxia, trauma, anoxia-ischemia and water infusion.

A. Brain-Water Content Determinations in the Normal Animal Using Two Methods

1. "Classical" Procedure

Using the "constant-weight" approach, temperatures ranging from 110° to 116° C and time intervals of over 98 hours, the mean brain-water content of four groups of rats (16 animals) was found to be 78.411 per cent of the original fresh tissue weight (Table 1). The tabulated results may be seen below. The average body weight was approximately 481 gm and the average age about 13 months.

TABLE I TABULATED WHOLE-BRAIN WATER-CONTENT VALUES FOR ALL FOUR GROUPS OF RATS

Rat Group No.	Weight of rats in grams	Approximate per cent of "water" in rat brain (% of weight loss, mean of A and B)
1 (A and B)	491.5	78.793
2 (A and B)	479	78.615
3 (A and B)	467	78.069
4 (A and B)	485	78.168
Mean = 78.411 per cent		

When the percentage of weight loss, or the "brain-water content" was observed as a function of the time interval of dehydration, an increasing percentage of weight loss was detected as the period of drying increased. This trend, found in all four groups of brains, did not show any tendency to change even after 405 hours of dehydration. When all the values were pooled and a product-moment correlation test was done, using the time interval and the percentage of weight loss as variables, a correlation coefficient (r) of +0.97 was found. The time intervals ranged between 98 and 405 hours.

The differences in percentages in water content between opposite halves (A and B) of the brains were: 1.255 per cent for group 1, 0.254 per cent for group 2, 0.638 per cent for group 3, and 0.092 per cent for group 4. The mean A and B difference when all four groups were pooled was 0.560 per cent. The range of mean brain water content values for all four groups (also expressed in percentages of their fresh tissue weights) was 0.724 per cent. Thus, this much variation was found in a similar, all-male rat population where the weight range was only of the order of 24.5 grams.

The tabulated mean values for the percentages of weight loss against the length of times of dehydration for each group of rats as well as for the whole population are presented in Tables 2, 3, 4, 5, 6 and in Figures 1, 2, 3. Graphs corresponding to each set of data are also shown. In Figure 3, the best-fitting straight line was found by regression. In Tables 2, 3, 4, 5 the first figures in each "per cent of weight loss" column, i.e., at 98 hours, represent "constant-weight"

values. These figures are the means of the first two consecutive weighings within 0.00025 grams and, as such, only these means have been recorded.

Tables 2 through 5 give the actual values for each group of rats, separating the figures into opposite brain halves A and B. At the bottom of each table the mean per cent of weight loss for the composite rat group is given.

Most of the presented observations and those to follow were done using this group separation procedure in order to facilitate the keeping and analyzing of data. The following is an example of the way any single set of values was compiled.

The first figure shown in the column labeled Mean Weight of Tissues for the rats in Group 1A (Table 2) is given as 0.68055. The amounts actually recorded, however, were:

0.66350+ grams
0.69794
0.67865
0.68212
2.72221 grams

The mean for these four values was approximately 0.68055 (2.722221 ÷ 4 = 0.680552). This last figure was recorded and used for subsequent tabulations and statistical analysis, being considered as one independent mean value.

The formulas used to find the straight line and the correlation coefficient in Figure 3 (161) were:

FIGURE 1

PER CENT WEIGHT LOSS IN WHOLE RAT BRAIN

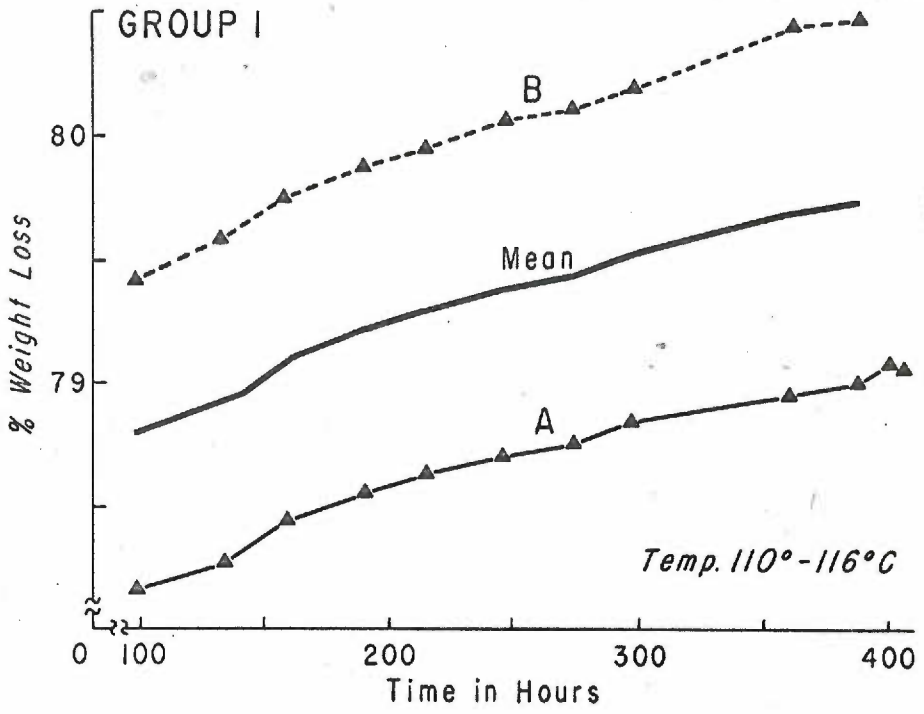
VERSUS TIME OF DEHYDRATION

Each of the following four graphs (Figures 1 and 2) corresponds to one group of animals (four rats) where the percentage of weight loss from the original fresh tissue weight is plotted against the time of dehydration in hours. As stated previously, the letters A and B correspond to opposite brain halves. The mean values for each group has also been plotted. The temperature as indicated in the graphs ranged between 110° C and 116° C.

GROUP I (Table 2).

GROUP 2 (Table 3).

% WGT. LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION



% WGT. LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION

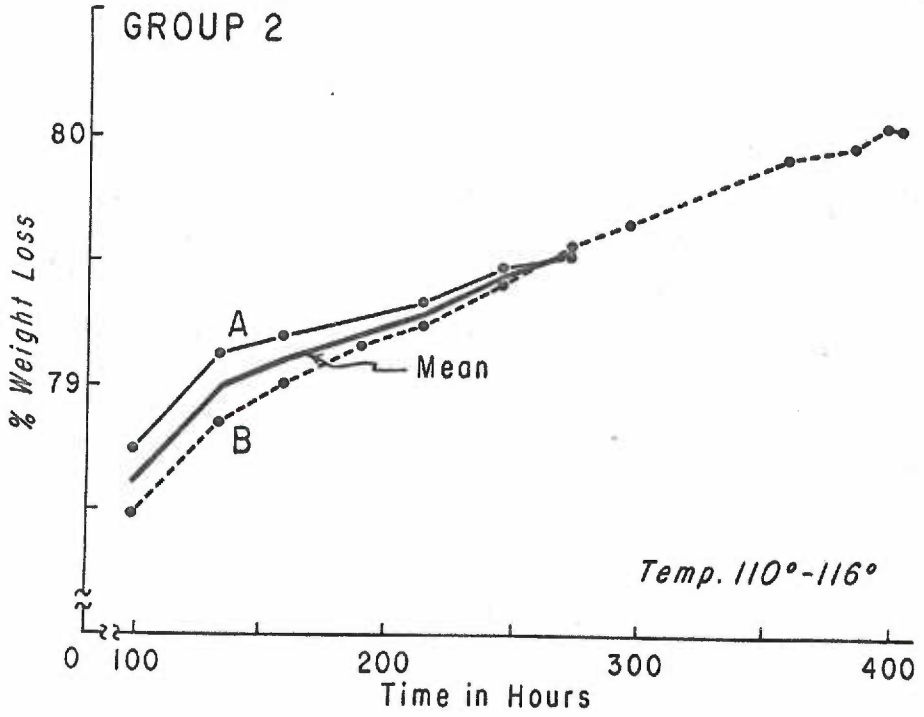


FIGURE 2

PER CENT WEIGHT LOSS IN WHOLE RAT BRAIN

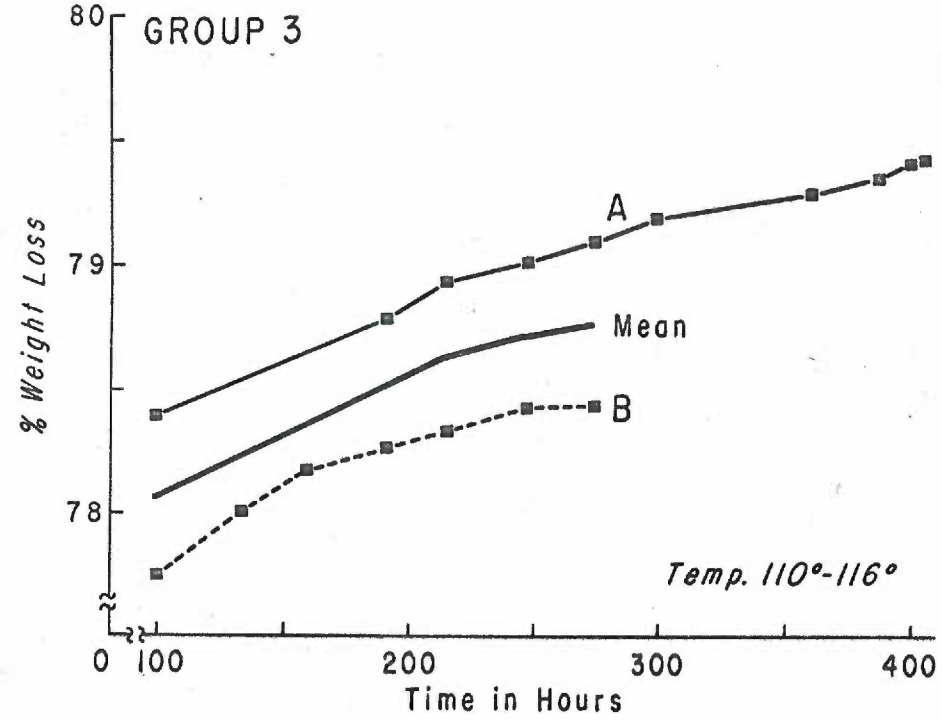
VERSUS TIME OF DEHYDRATION

For details of these graphs see the legend of Figure 1 on previous page.

GROUP 3 (Table 4).

GROUP 4 (Table 5).

% WGT. LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION



% WGT. LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION

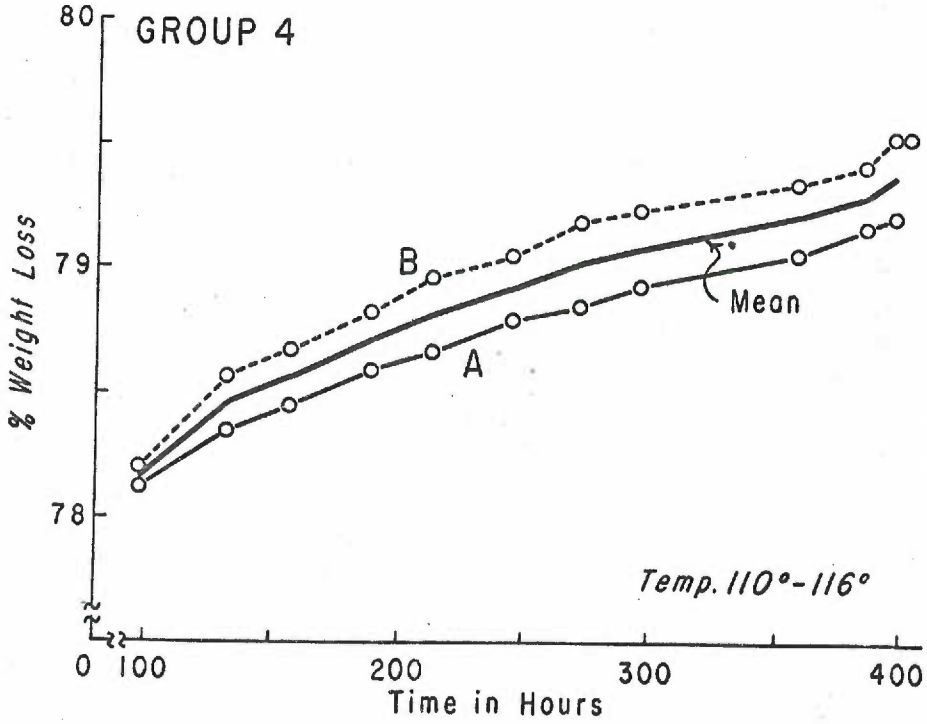


TABLE II TABULATED MEAN VALUES FOR PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION. (Includes observations for Group 1A and 1B and for Mean of 1A and 1B)

GROUP 1A

Time Spent in Oven-in Hours	Mean Weight of Tissue-in Grams	Weight of Tissue in % of Wet Weight	% of Weight Loss
0	0.68055	100	0
98	0.14860	21.835	78.165
133	0.14785	21.725	78.275
158	0.14670	21.556	78.444
190	0.14595	21.446	78.554
214	0.14545	21.372	78.628
246	0.14490	21.292	78.708
273	0.14460	21.248	78.752
297	0.14400	21.159	78.841
360	0.14330	21.056	78.944
387	0.14300	21.012	78.988
399	0.14245	20.932	79.068
405	0.14255	20.946	79.054

GROUP 1B

0	0.63260	100	0
98	0.13015	20.580	79.420
133	0.12910	20.414	79.586
158	0.12810	20.256	79.744
190	0.12725	20.122	79.878
214	0.12565	20.059	79.941
246	0.12610	19.940	80.060
273	0.12560	19.892	80.108
297	0.12525	19.806	80.194
360	0.12375	19.566	80.432
387	0.12350	19.529	80.471

GROUP 1A and 1B - MEAN

0	0
98	78.793
133	78.931
158	79.094
190	79.216
214	79.285
246	79.384
273	79.43
297	79.518
360	79.688
387	79.730

TABLE III TABULATED MEAN VALUES FOR PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION. (Includes observations for Group 2A and 2B, and for the Mean of 2A and 2B)

GROUP 2A

Time Spent in Oven-in Hours	Mean Weight of Tissue-in Grams	Weight of Tissue in % of Wet Weight	% of Weight Loss
0	0.62635	100	0
98	0.13315	21.258	78.742
133	0.13070	20.867	79.133
158	0.13030	20.803	79.197
214	0.12950	20.675	79.325
246	0.13860	20.532	79.468
273	0.12840	20.500	79.500

GROUP 2B

0	0.67150	100	0
98	0.14445	21.512	78.488
133	0.14205	21.154	78.846
158	0.14095	20.990	79.010
190	0.14000	20.849	79.151
214	0.13935	20.752	79.248
246	0.13825	20.598	79.412
273	0.13725	20.439	79.561
297	0.13665	20.350	79.650
360	0.13480	20.074	79.926
387	0.13445	20.022	79.978
399	0.13400	19.955	80.045
405	0.13405	19.963	80.037

GROUP 2A and 2B - MEAN

0	0
98	78.615
133	78.990
158	79.104
214	79.287
246	79.440
273	79.531

TABLE IV TABULATED MEAN VALUES FOR PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION. (Includes observations for Group 3A and 3B, and for the Mean of 3A and 3B)

GROUP 3A

Time Spent in Oven-in Hours	Mean Weight of Tissue-in Grams	Weight of Tissue in % of Wet Weight	% of Weight Loss
0	0.67790	100	0
98	0.14650	21.611	73.387
190	0.14385	21.220	78.780
214	0.14280	21.065	78.935
246	0.14225	20.984	79.016
273	0.14170	20.903	79.097
297	0.14110	20.814	79.186
360	0.14035	20.704	79.296
387	0.14000	20.652	79.348
399	0.13955	20.586	79.414
405	0.13945	20.571	79.429
GROUP 3B			
0	0.62830	100	0
98	0.13980	22.251	77.749
133	0.13815	21.988	78.012
158	0.13715	21.829	78.171
190	0.13655	21.733	78.267
214	0.13610	21.662	78.338
246	0.13550	21.574	78.426
273	0.13550	21.566	78.434
GROUP 3A and 3B - MEAN			
0			0
98			78.069
190			78.524
214			78.636
246			78.721
273			78.766

TABLE V TABULATED MEAN VALUES FOR PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION. (Includes observations for Group 4A and 4B, and for the Mean of 4A and 4B)

GROUP 4A

Time Spent in Oven-in Hours	Mean Weight of Tissue-in Grams	Weight of Tissue in % of Wet Weight	% of Weight Loss
0	0.63945	100	0
98	0.13990	21.878	78.122
133	0.13850	21.659	78.341
158	0.13780	21.550	78.450
190	0.13685	21.401	78.599
214	0.13635	21.323	78.677
246	0.13525	21.214	78.786
273	0.13525	21.151	78.849
297	0.13475	21.073	78.027
360	0.13385	20.932	79.068
387	0.13315	20.823	79.177
399	0.13295	20.791	79.209

GROUP 4B

0	0.63160	100	0
98	0.13760	21.786	78.214
133	0.13540	21.438	78.562
158	0.13470	21.327	78.673
190	0.13375	21.176	78.824
214	0.13285	21.034	78.966
246	0.13230	20.947	79.053
273	0.13150	20.820	79.180
297	0.13120	20.773	79.227
360	0.13010	20.645	79.355
387	0.12970	20.598	79.402
399	0.12925	20.464	79.536
405	0.12925	20.464	79.536

GROUP 4A and 4B - MEAN

0	0
98	78.168
133	78.452
158	78.562
190	78.712
214	78.822
246	78.920
273	79.015
297	79.077
360	79.212
387	79.290
399	79.373

FIGURE 3

PER CENT WEIGHT LOSS IN WHOLE RAT BRAIN

VERSUS TIME OF DEHYDRATION

The graph corresponds to all four groups of animals (16 rats) whose mean values have been pooled (Table 6). Again the percentage of weight loss from the original fresh tissue weight is plotted against the time of dehydration in hours. The values for each group are plotted as shown on the graph key. The best-fitting straight line is shown together with the correlation coefficient (r). (For formulas used, see pertinent section in text that follows.)

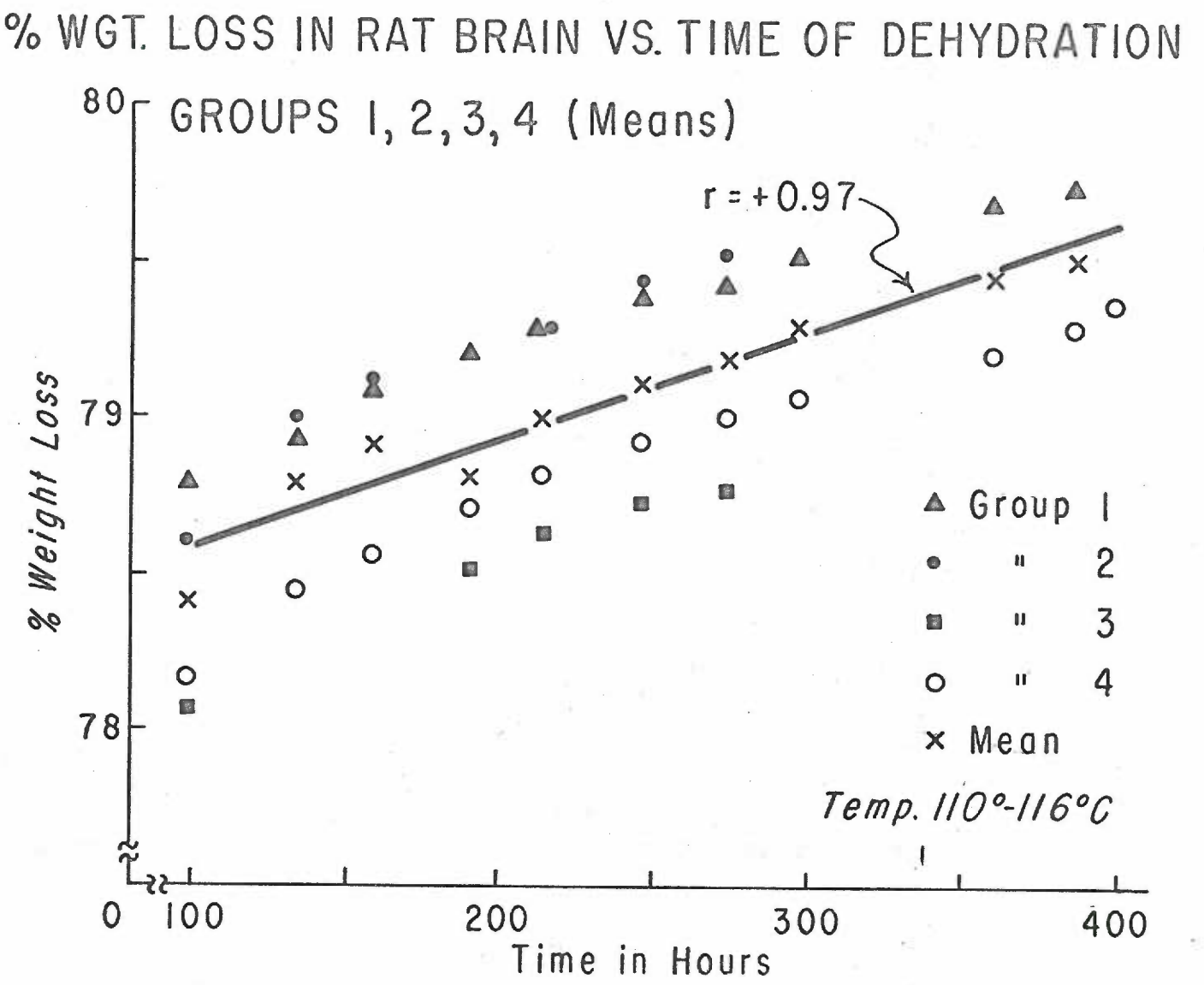

% WGT. LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION
GROUPS 1, 2, 3, 4 (Means)
r = +0.97
% Weight Loss
80
79
78
0
100
200
300
400
Time in Hours
Group 1
" 2
" 3
" 4
Mean
Temp. 110°-116°C

TABLE VI POOLED DATA FROM ALL FOUR GROUPS INDICATING THE MEAN PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION (OR TIME SPENT IN OVEN)

Time Spent in Oven in Hours	Mean Per Cent of Weight Loss
0	0
98	78.411
133	78.791
158	78.920
190	78.817
214	79.008
246	79.116
273	79.186
297	79.298
360	79.450
387	79.510

1. For the Product-Moment Correlation (Correlation Coefficient):

$$r = \frac{N\Sigma XY - \Sigma X\Sigma Y}{\sqrt{[N\Sigma X^2 - (\Sigma X)^2][N\Sigma Y^2 - (\Sigma Y)^2]}}$$

2. Regression to find a straight line:

$Y = Bx + C$ where

$$B = \frac{N\Sigma XY - \Sigma X\Sigma Y}{N\Sigma X^2 - (\Sigma X)^2}$$

and $$C = \frac{\Sigma X^2\Sigma Y - X\ XY}{N\Sigma X^2 - (\Sigma X)^2}$$

2. Modification of the "Classical" Procedure

In obtaining the brain-water content of both hemispheres (A and B) for each of the rat groups, two sets of criteria were used. In the first place the weight measurements selected for determining the mean had to be three of more consecutive figures whose numerical difference was less than 0.00025 grams. In many instances this difference was only a very small fraction of this amount. Since the tissues were being weighed repeatedly after almost every hour of dehydration, little or no difficulty was encountered in reaching this accuracy. If a set of data did not fulfill these requirements, the values chosen were those which, because of their own relative similarity and their difference from the other figures, fell into the same "category". The time interval wherein the two sets of criteria were met turned out to be in most cases between 14 and 19 hours. This interval corresponded to a "plateau"

identifiable in the graph of Figure 6 which relates the time of dehydration to the percentage of weight loss.*

Therefore when brain dehydration was carried out at temperatures ranging from 91° to 93° C, the mean brain-water content of four groups of rats (a total of 16) was found to be 77.783 per cent of the original fresh tissue weight. The tabulated results may be seen below. The average body weight was approximately 394 grams and the age about 410 days (i.e., 13 to 14 months).

TABLE VII TABULATED WHOLE-BRAIN WATER-CONTENT VALUES FOR ALL FOUR GROUPS OF RATS

Rat Group No.	Weight of rats in grams	Approximate per cent of "water" in rat brain (per cent of weight loss, mean of A and B)
5 (A and B)	367	78.153
6 (A and B)	310	77.755
7 (A and B)	436	77.875
8 (A and B)	462	77.349

Mean = 77.783 per cent.

Again, when the percentage of weight loss (or "brain-water content") was observed as a function of the period of dehydration, continuous weight loss on repeated heating was the rule. Thus, by 147 hours the tissues were still losing weight and showed no tendency to approach a "real" constant weight.

*The figures referred to here have been identifed by brackets in Tables 8 through 11. The mean for each of those sets of values yielded the percentage of brain water content for each group (either A or B). Opposite halves were then combined in order to obtain the mean values for the specific group (A and B).

The differences in percentages in water content between opposite halves (A and B) of the brains were: 0.331 per cent for group 5, 0.085 per cent for group 6, 0.749 per cent for group 7, and 0.024 per cent for group 8. The mean A and B difference when all four groups were pooled together was 0.297 per cent. The range of mean brain-water content values for all four groups (when expressed in percentages of their fresh tissue weights), was 0.804 per cent, while the mean body weight range in the same rat population was 152 grams.

In the next pages both tables and their representative graphs are shown (see Tables 8 - 11 and Figures 4, 5); also, the pooled data for groups 5 through 8, together with their graphical ;equivalent is presented (Table 12, Figure 6). In each graph, a "flattening out" (or plateau) appears immediately after the area of accelerated weight loss (relative to the period of drying) and can be identified. This "plateau" precedes the area of "irregular tissue weight", which in Figure 6 lies between 20 and 35 hours and also precedes the region of continuous weight loss, which on the same Figure begins at 35 hours.

FIGURE 4

PER CENT WEIGHT LOSS IN WHOLE RAT BRAIN

VERSUS TIME OF DEHYDRATION

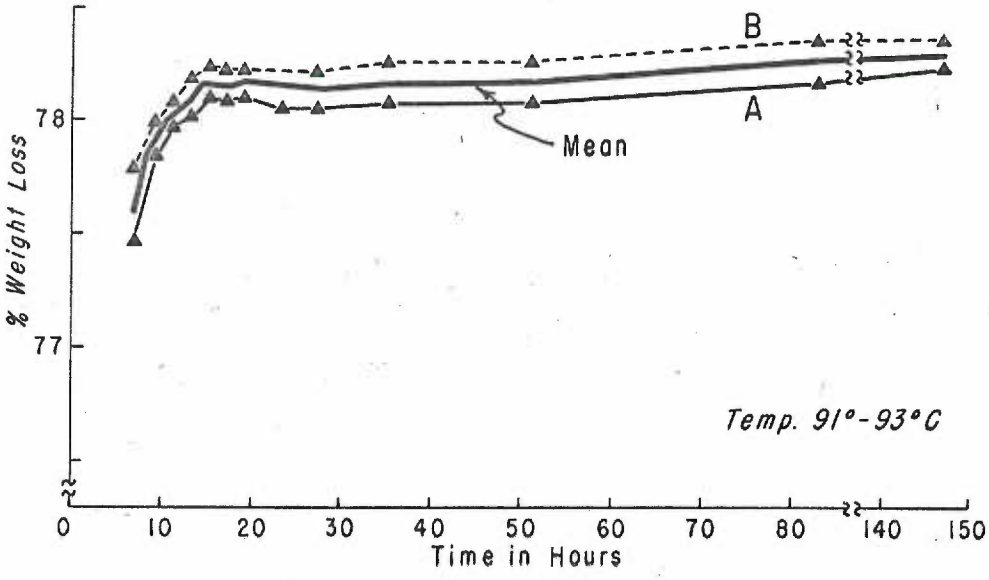
Each of the following four graphs (Figures 4 and 5) corresponds to one group of animals (4 rats) where the percentage of weight loss from the original fresh tissue weight is plotted against the time of dehydration in hours. As stated previously, the letters A and B correspond to opposite brain halves. The mean values for each group have also been plotted. The temperature as indicated in the graphs ranged between 91° and 93° C.

GROUP 5 (Table 8).

GROUP 6 (Table 9).

% WEIGHT LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION

GROUP 5



% WEIGHT LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION

GROUP 6

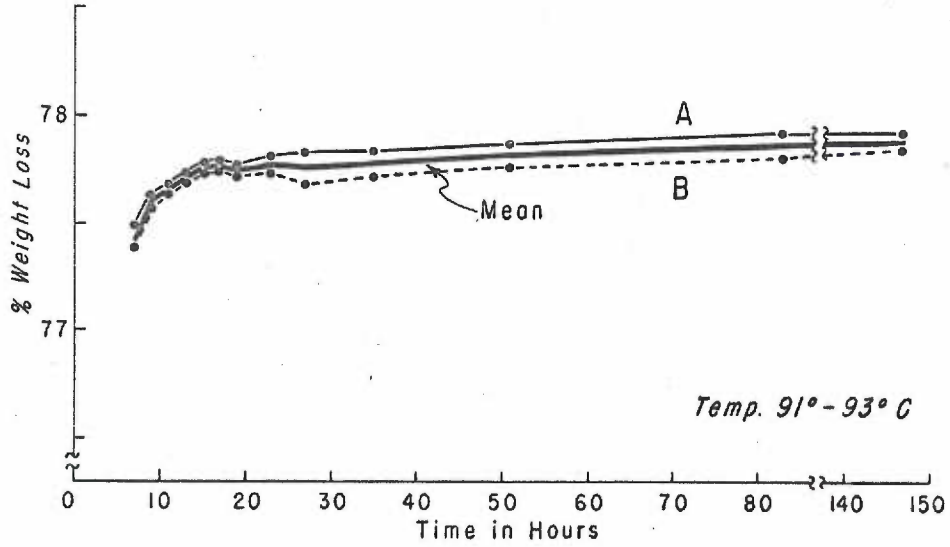


FIGURE 5

PER CENT WEIGHT LOSS IN WHOLE RAT BRAIN

VERSUS TIME OF DEHYDRATION

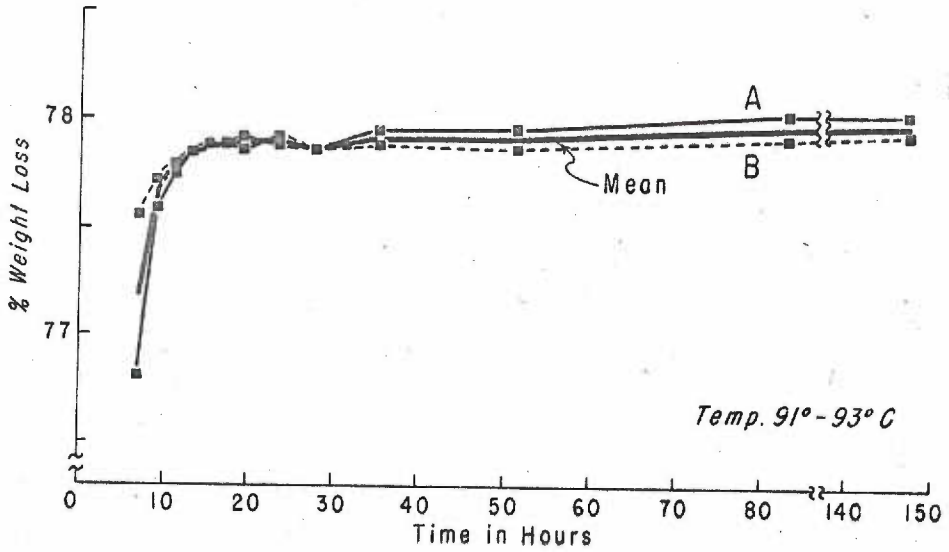
For details of these graphs see legend of Figure 4 on previous page.

GROUP 7 (Table 10).

GROUP 8 (Table 11).

% WEIGHT LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION

GROUP 7



% WEIGHT LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION

GROUP 8

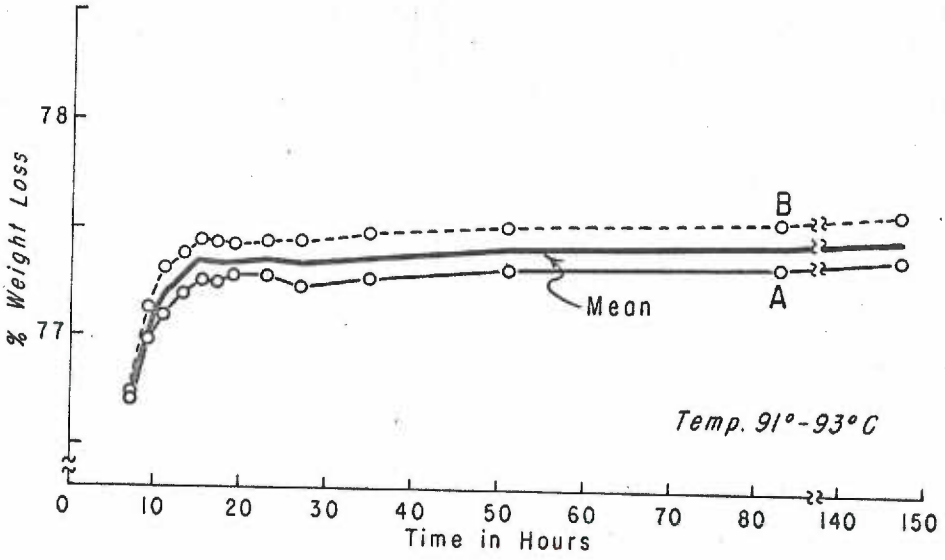


TABLE VIII TABULATED MEAN VALUES FOR PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION. (Includes observations for Group 5A and 5B and for the Mean of 5A and 5B.)

GROUP 5A

Time Spent in Oven-in Hours	Mean Weight of Tissue-in Grams	Weight of Tissue in % of Wet Weight	% of Weight Loss
0	0.54912	100	0
7	0.12377	22.540	77.460
8	0.12198	22.214	77.786
9	0.12166	22.155	77.845
10	0.12107	22.048	77.952
11	0.12094	22.024	77.976
12	0.12078	21.995	78.005
13	0.12071	21.982	78.018
14	0.12034)	21.915	78.085)
15	0.12034)	21.915	78.085)
17	0.12035)	21.917	78.083)
19	0.12022	21.893	78.107
23	0.12051	21.946	78.054
27	0.12050	21.944	78.056
35	0.12043	21.931	78.069
51	0.12039	21.924	78.076
83	0.11994	21.842	78.158
147	0.11956	21.773	78.227

GROUP 5B

0	0.62480	100	0
7	0.13876	22.209	77.791
8	0.13797	22.082	77.918
9	0.13760	22.023	77.977
10	0.13723	21.964	78.036
11	0.13694	21.917	78.083
12	0.13672	21.882	78.118
13	0.13634	21.821	78.179
14	0.13614)	21.789	78.211)
15	0.13600)	21.767	78.233)
17	0.13608)	21.780	78.220)
19	0.13608)	21.780	78.220)
27	0.13620	21.799	78.201
35	0.13594	21.757	78.243
51	0.13588	21.748	78.252
83	0.13530	21.655	78.345
147	0.13533	21.660	78.340

(Concluded on next page.)

TABLE VIII (Concluded)

GROUP 5A and 5B - MEAN

Time Spent in Oven in Hours	Mean Per Cent of Weight Loss
0	0
7	77.626
8	77.852
9	77.911
10	77.994
11	78.030
12	78.062
13	78.099
14	78.148
15	78.159
17	78.152
19	78.164
23	78.054
27	78.129
35	78.156
51	78.164
83	78.252
147	78.284

TABLE IX TABULATED MEAN VALUES FOR PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION. Includes observations for Group 6A, 6B and for the Mean of 6A and 6B.)

GROUP 6A

Time Spent in Oven-in Hours	Mean Weight of Tissue-in Grams	Weight of Tissue in % of Wet Weight	% of Weight Loss
0	0.62833	100	0
7	0.14152	22.523	77.477
8	0.14095	22.432	77.568
9	0.14062	22.380	77.620
10	0.14036	22.339	77.661
11	0.14031	22.331	77.669
12	0.14011	22.299	77.701
13	0.13992	22.269	77.731
14	0.13968)	22.230	77.770)
15	0.13968)	22.230	77.770)
17	0.13963)	22.222	77.778)
19	0.13972)	22.237	77.763)
23	0.13944	22.192	77.808
27	0.13936	22.179	77.821
35	0.13932	22.173	77.827
51	0.13910	22.138	77.862
83	0.13882	22.093	77.907
147	0.13882	22.093	77.907
GROUP 6B			
0	0.61407	100	0
7	0.13883	22.608	77.392
8	0.13804	22.480	77.520
9	0.13769	22.423	77.577
10	0.13737	22.370	77.630
11	0.13737	22.370	77.630
12	0.13716	22.336	77.664
13	0.13695	22.302	77.698
14	0.13668)	22.258	77.742)
15	0.13674)	22.268	77.732)
17	0.13666)	22.255	77.745)
19	0.13681	22.279	77.721
23	0.13681	22.279	77.721
27	0.13704	22.317	77.683
35	0.13686	22.287	77.713
51	0.13666	22.255	77.745
83	0.13636	22.206	77.794
147	0.13607	22.159	77.841

(Concluded on next page.)

TABLE IX (Concluded)

GROUP 6A and 6B - MEAN

Time Spent in Oven in Hours	Mean Per Cent of Weight Loss
0	0
7	77.435
8	77.544
9	77.599
10	77.646
11	77.650
12	77.683
13	77.715
14	77.756
15	77.751
17	77.762
19	77.742
23	77.765
27	77.752
35	77.770
51	77.804
83	77.851
147	77.874

TABLE X TABULATED MEAN VALUES FOR PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION. (Includes observations for Group 7A, 7B and Mean of 7A and 7B.)

GROUP 7A

Time Spent in Oven-in Hours	Mean Weight of Tissue-in Grams	Weight of Tissue in % of Wet Weight	% of Weight Loss
0	0.72101	100	0
7	0.16717	23.186	76.814
8	0.16424	22.779	77.221
9	0.16155	22.406	77.594
10	0.16058	22.272	77.728
11	0.16041	22.248	77.752
12	0.16014	22.211	77.789
13	0.15973	22.154	77.846
14	0.15962	22.138	77.862
15	0.15947)	22.118	77.882)
17	0.15946)	22.116	77.884)
19	0.15921	22.082	77.918
23	0.15940	22.108	77.892
27	0.15962	22.138	77.862
35	0.15896	22.047	77.953
51	0.15894	22.044	77.956
83	0.15834	21.961	78.039
147	0.15843	21.973	78.027

GROUP 7B

0	0.64612	100	0
7	0.14497	22.437	77.563
8	0.14439	22.347	77.653
9	0.14392	22.275	77.725
10	0.14354	22.216	77.784
11	0.14358	22.222	77.778
12	0.14333	22.185	77.817
13	0.14312)	22.151	77.849)
14	0.14312)	22.151	77.849)
15	0.14291)	22.118	77.882)
17	0.14286)	22.114	77.886)
19	0.14299)	22.131	77.869)
23	0.14278	22.098	77.902
27	0.14297	22.127	77.873
35	0.14287	22.112	77.888
51	0.14294	22.123	77.877
83	0.14264	22.076	77.924
147	0.14254	22.061	77.939

(Concluded on next page)

TABLE X (Concluded)

GROUP 7A and 7B - MEAN

Time Spent in Oven-in Hours	% of Weight Loss
0	0
7	77.189
8	77.437
9	77.660
10	77.756
11	77.765
12	77.803
13	77.848
14	77.856
15	77.882
17	77.885
19	77.894
23	77.897
27	77.868
35	77.921
51	77.917
83	77.982
147	77.983

TABLE XI TABULATED MEAN VALUES FOR PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION. (Includes observations for Group 8A, 8B and Mean of 8A and 8B.)

GROUP 8A

Time Spent in Oven-in Hours	Mean Weight of Tissue-in Grams	Weight of Tissue in % of Wet Weight	% of Weight Loss
0	0.62838	100	0
7	0.14634	23.288	76.712
8	0.14525	23.115	76.885
9	0.14460	23.012	76.988
10	0.14423	22.953	77.047
11	0.14382	22.887	77.113
12	0.14349	22.835	77.165
13	0.14323	22.794	77.206
14	0.14297)	22.752	77.248)
15	0.14290)	22.741	77.259)
17	0.14292)	22.744	77.256)
19	0.14274)	22.716	77.284
23	0.14276	22.719	77.281
27	0.14303	22.762	77.238
35	0.14278	22.722	77.278
51	0.14249	22.676	77.324
83	0.14238	22.658	77.342
147	0.14217	22.625	77.375

GROUP 8B

0	0.61219	100	0
7	0.14242	23.264	76.736
8	0.14100	23.032	76.968
9	0.14007	22.880	77.120
10	0.13925	22.746	77.254
11	0.13889	22.687	77.313
12	0.13880	22.673	77.327
13	0.13850	22.624	77.376
14	0.13829	22.589	77.411
15	0.13808)	22.555	77.445)
17	0.13810)	22.558	77.442)
19	0.13818)	22.571	77.429)
23	0.13806)	22.555	77.445)
27	0.13802)	22.545	77.455)
35	0.13780	22.509	77.491
51	0.13762	22.480	77.520
83	0.13740	22.444	77.556
147	0.13715	22.403	77.597

(Concluded on next page)

TABLE XI (Concluded)

GROUP 8A and 8B - MEAN

Time Spent in Oven-in Hours	% of Weight Loss
0	0
7	76.724
8	76.927
9	77.054
10	77.151
11	77.213
12	77.246
13	77.291
14	77.330
15	77.352
17	77.349
19	77.357
23	77.363
27	77.347
35	77.385
51	77.422
83	77.449
147	77.486

FIGURE 6

PER CENT WEIGHT LOSS IN WHOLE RAT BRAIN

VERSUS TIME OF DEHYDRATION

The graph includes all four groups of animals (16 rats) studied by the modified technique of the classical procedure for Brain Water Content Determination (Groups 5, 6, 7, 8. Table 12). Again, the percentage of weight loss from the original fresh tissue weight is plotted against the time of dehydration in hours. The values for each group are plotted as shown on the graph key.

% WEIGHT LOSS IN RAT BRAIN VS. TIME OF DEHYDRATION

GROUPS 5, 6, 7, 8 (Means)

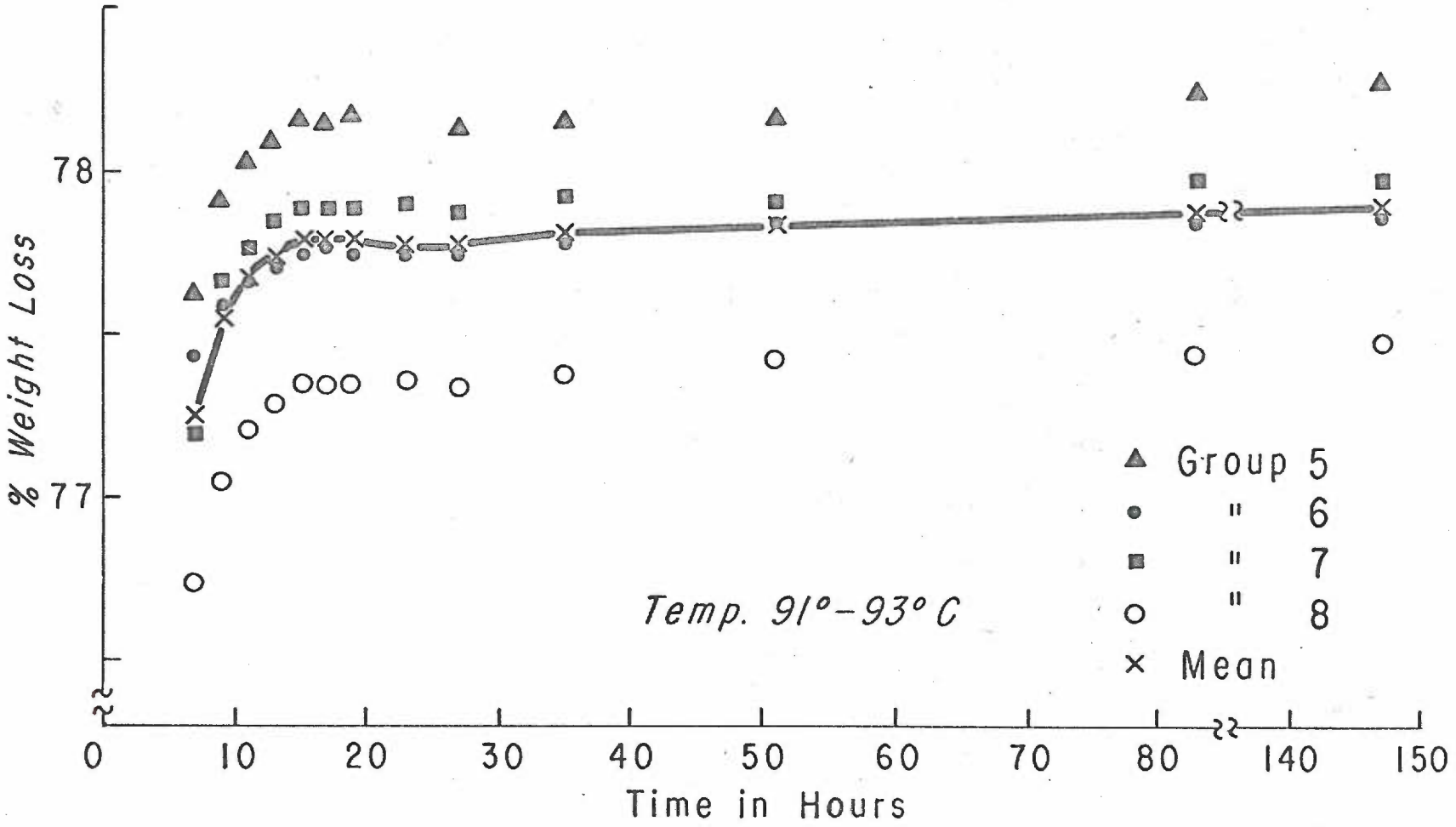


TABLE XII. POOLED DATA FROM ALL FOUR GROUPS INDICATING MEAN PER CENT OF WEIGHT LOSS VERSUS TIME OF DEHYDRATION

Time Spent in Oven-in Hours	% of Weight Loss
0	0
7	77.244
8	77.440
9	77.556
10	77.637
11	77.665
12	77.699
13	77.738
14	77.773
15	77.786
17	77.787
19	77.789
23	77.770
27	77.774
35	77.808
51	77.827
83	77.884
147	77.907

THE EXPERIMENTAL PRODUCTION OF CEREBRAL EDEMA BY THE USE OF TRIETHYL TIN ACETATE.

In the process of producing and observing cerebral edema, parameters other than those dealing directly with the brain substance were also studied. Some of these such as body weight provide some idea of the relative toxicity of the compound used. They also document a comparable phenomenon which occured in those patients originally poisoned with Stalinon® (1). Other parameters like symptomatology and the nature, onset and course of the disability relate more clearly with the basic pathophysiologic process which has been investigated in this study.

B. Symptomatology and Associated Body Changes in Triethyl Tin Acetate (TETA)-treated Animals.

1. Acutely treated animals.

Of the 28 animals injected with TETA (10 mg/kg BW), 27 were alive 48 hours later; the one rat having died during the second night. All animals however developed a characteristic sequence of symptoms noticeable throughout the duration of the experiment.

Within one-half hour after intraperitoneal injection with TETA there was a generalized cramping of the skeletal musculature of the animal which was most noticeable from the abdomen down. This was followed by a picture of generalized weakness with flaccid paralysis and dragging of the hind limbs and, later, by prostration. Quite noticeable also was the hypothermia. By two to four hours after injection

the rats seemed to recover slightly; however, by 24 hours there was again marked lethargy, listlessness and signs of an increasing generalized weakness. At about this time too, secretion of red (bloody) tears was observed.

The rats, even though quite prostrated, attempted to eat and drink until they were sacrificed. Nevertheless there was an average of 27.7 grams of weight loss by 48 hours which corresponded to about 13.8 per cent of their original total body weight.

By 48 hours the picture was similar to that seen at 24 hours, except for an increase in the severity of the same. As previously stated the pattern and sequence of events just described was uniformly the same in each animal treated, except for the animal which died prior to 48 hours. In this case the same order of events took place within a shorter period of time. Some of the physical changes just described may be seen in Figure 7A, where the experimental animal is contrasted to a healthy control.

While dissecting the brains of the treated animals, it was possible to easily differentiate these from the normals by a series of striking features present in the former. In the edematous brains there was a plastering of the brain substance against the calvarium which together with its mushy consistency made it relatively difficult for the experimenter to separate it from its bony encasings. Ventrally, a crowding of the cerebral hemispheres against the optic chiasm, tuber cinereum and mammillary body resulted in partial displacement or impingement of the internal carotid and posterior communicating arteries (Figure 12B and 12D).

2. Chronically treated animals.

As noted in the Methods and Materials section, two separate studies of chronic TETA intoxication were conducted (the second study having been undertaken to provide specimens for histological examination). In both cases the symptomatology and associated body changes followed parallel courses; only the first study will be completely discussed here.

Of the 40 animals given TETA in their food and water, only 30 were alive by the 12th day. Of these, 28 were selected for the various gravimetric and electrolyte determinations.

The first noticeable effect of TETA in the experimental animals was a reduction in the amount eaten and drunk with a consequent cessation of growth and loss of weight. To a certain degree this was also true of the pair-fed animals, however the loss of body weight was not as extreme.

No other symptoms or signs were evident until the eighth day when "bloody" tears and petechiae were seen around the animals' nostrils and snouts. Also, at about this time, the first neurological symptoms appeared in the form of difficulty in manipulating their hind limbs, lethargy and listlessness. By the 10th day atrophy of the lower part of the trunk and hind limbs became quite obvious and was associated with the progressive flaccid paralysis observed in the same regions of the body. The animals' condition then deteriorated rapidly with the muscular flaccidity extending to the fore limbs. Finally, weakness forced the animals to remain motionless on their sides, unable to feed

themselves (Figures 7B, 8A, 8B, 9A and 9B). Thus, by the end of the experiment these chronically treated animals had lost an average of 46.1 grams (31.6 per cent decrease from their original body weight) as compared to the nontreated pair-fed rats which had gained 6.5 grams (4.4 per cent increase from their original body weight). The healthy control animals allowed to eat ad lib had developed normally and had gained 87.1 grams which represented a gain of 58.1 per cent in their total body weight.

In the second study the experimental animals also given TETA in their food and water lost 44.6 grams (a decrease of 30.5 per cent in body weight) while the non-treated pair-feds lost 9.5 grams (a decrease of 6.5 per cent from their original body weight). The ad lib controls developed normally and gained 81.2 grams, which represents a gain of 54.2 per cent in body weight.

These results are shown in Figures 10, 11 and Table 13. The effect of TETA on cessation of growth and loss of body weight is apparent. The loss of body weight seems to be beyond that explainable by a simple reduction in the amount of food consumed, as indicated by the less pronounced weight loss exhibited by the non-treated pair-fed animals.

While anesthetising the animals intraperitoneally prior to sacrifice, an almost complete anesthesia of the abdomen was noted. Once the skull of the animal was opened and the brain examined there was no possibility of confusing TETA treated animals with the controls. In the TETA treated animals the plastering of the brain substance against its bony encasings had apparently resulted in a certain degree of adhesion

of the meningeal membranes, making removal of the brain much more difficult. The brain had also a mushy consistency and there was a general crowding of the cerebral hemispheres against all the adjacent structures, most noticeably from the ventral view. The cerebral hemispheres, especially, encroached upon the optic chiasm, tuber cinereum, mamillary body and interpeduncular fossa (Figures 12A, 12B, 12C and 12D).

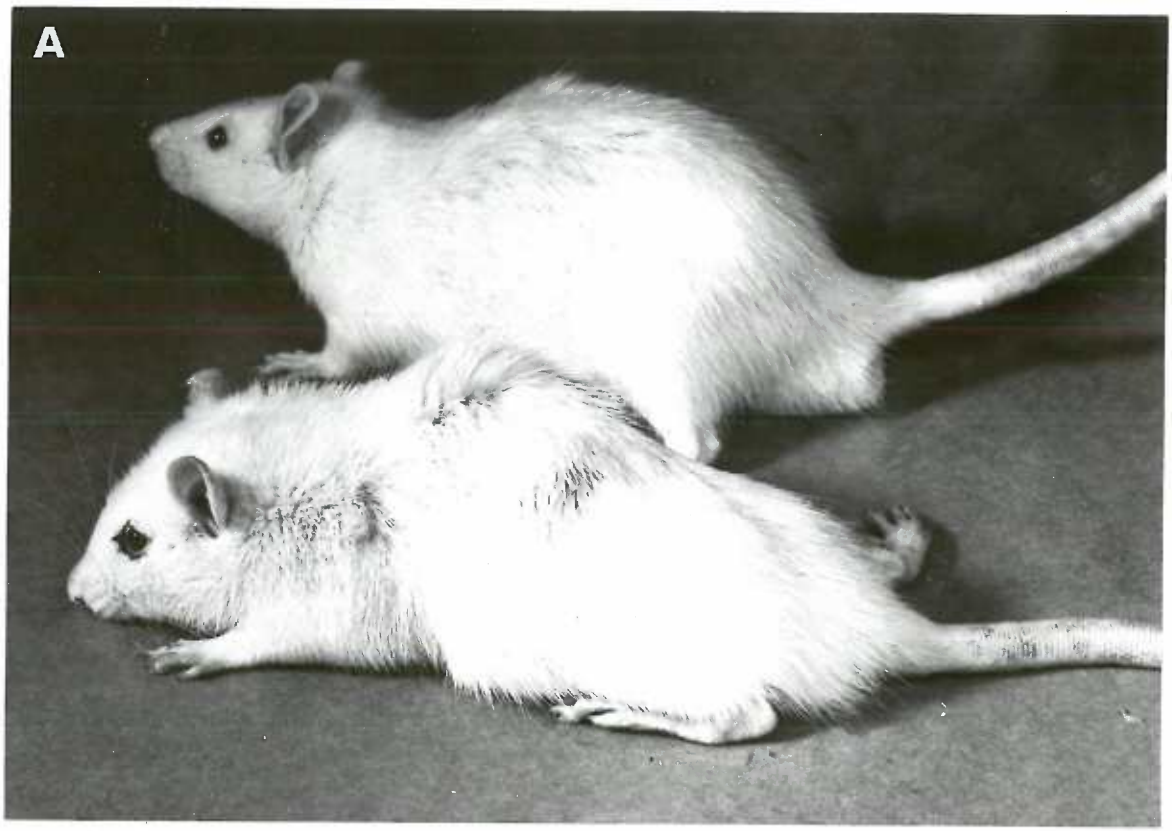
FIGURE 7

GROSS ANATOMICAL FEATURES

Comparison of animals treated with TETA versus normal controls.

A. A normal control rat (above) is pictured together with one which had been injected intraperitoneally 48 hours previously with TETA (10 mg/kg BW). Note in the animal below the flaccid position of its right hind limb, and apparent weakness of the other hind extremity (with consequent dragging of the caudal part of the body). Some degree of prostration and/or lethargy is also noticeable. "Bloody" tears are seen in the left periocular area.

B. Again, a normal control (above) is shown with an experimental chronically treated animal (below). This photograph was taken after 14 days of TETA ingestion. The marked prostration, cachexia, atrophy, flaccidity (most noticeably in the hind limbs) and general weakness are all evident.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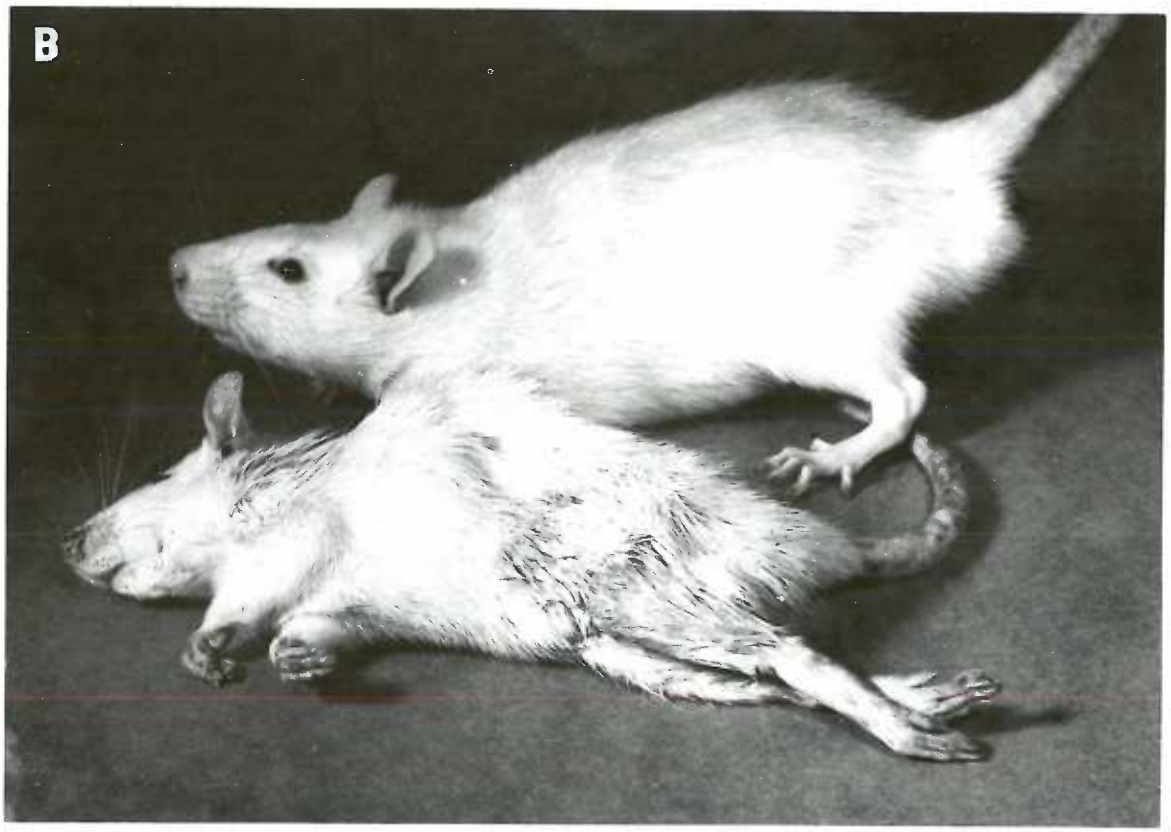
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FIGURE 8

GROSS ANATOMICAL FEATURES IN

CHRONICALLY TREATED RATS

A. Three animals with various degrees of pathology are presented. They all show atrophy of the caudal region of the body and flaccidity of the hind limbs. Varying degrees of prostration and weakness are also seen. Note that the animal on the far right shows a "band" in the abdomen which demarcates the area of extreme muscle wasting (below) from the region of lesser muscle atrophy and weight loss (above).

B. Flaccidity with complete loss of muscle power in the hind limbs of one of the experimental animals.

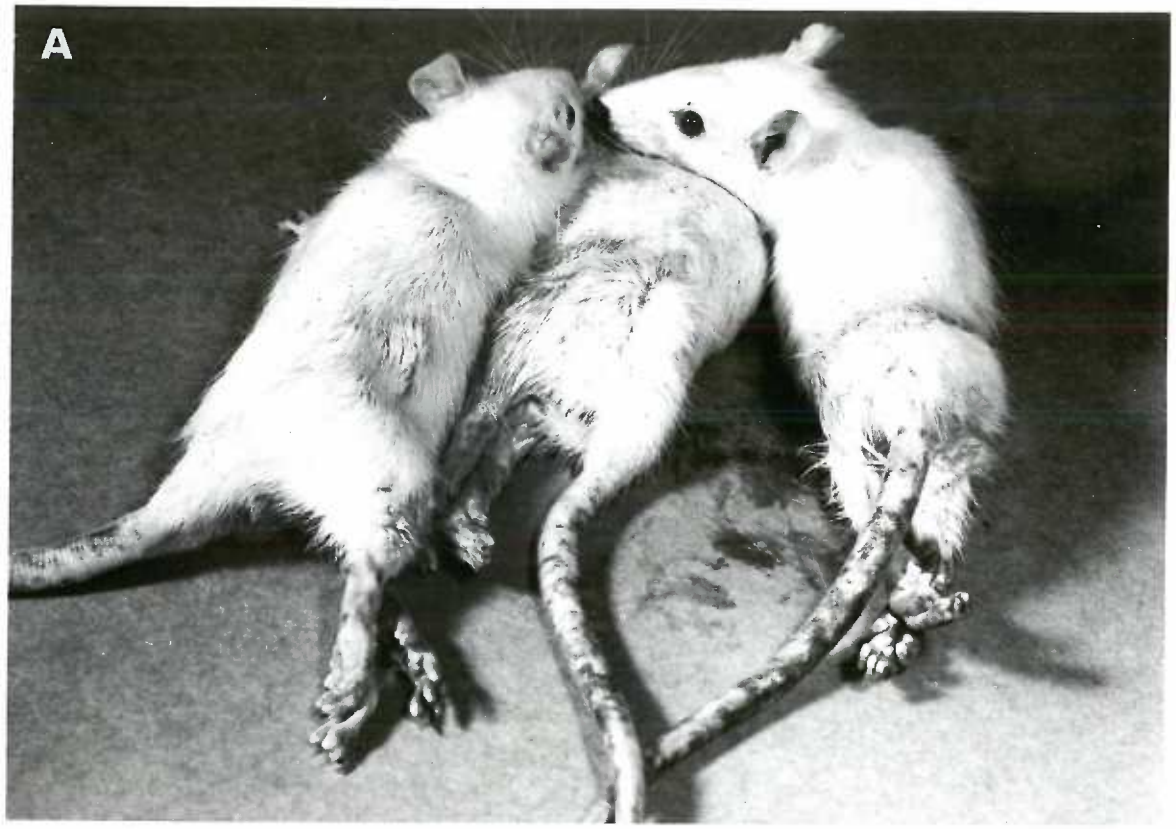
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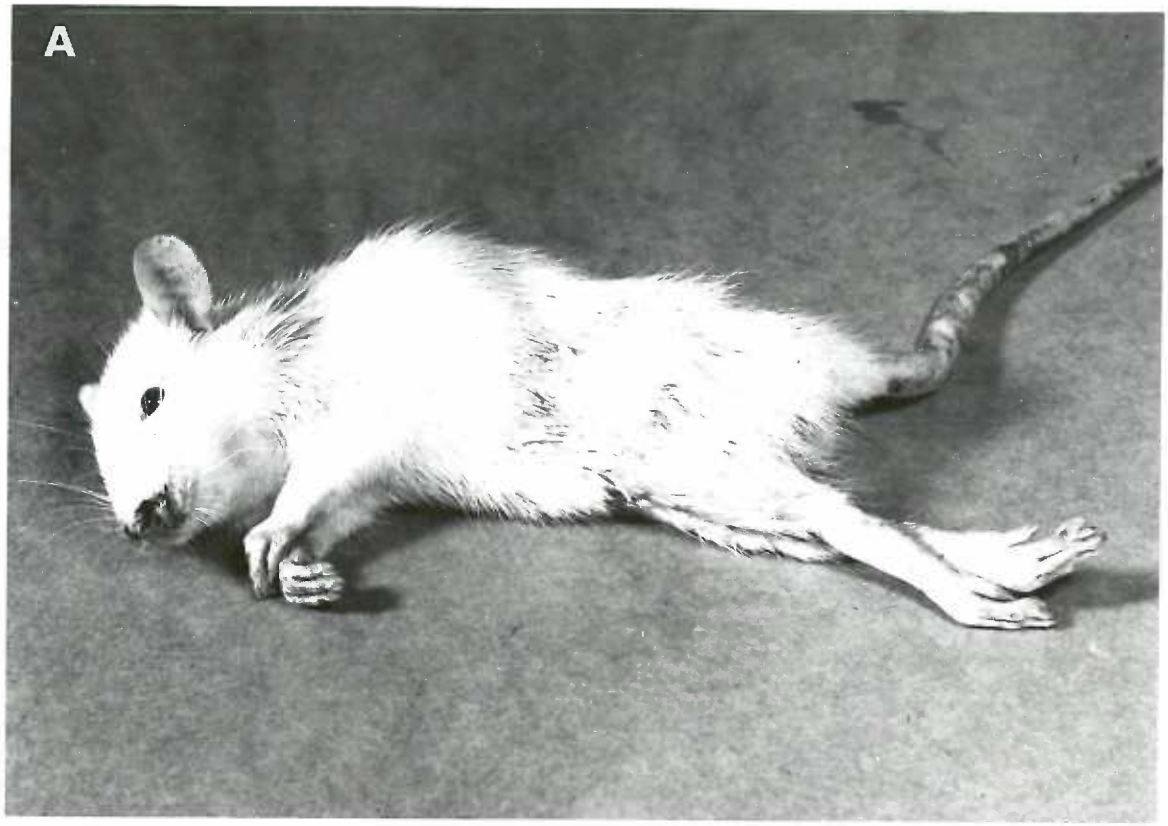
FIGURE 9

GROSS ANATOMICAL FEATURES IN

CHRONICALLY TETA TREATED RATS

A. This photograph demonstrates the marked prostration, flaccidity of the extremities and extreme muscle wasting (more accentuated in the hind limbs). At this stage, the animal was unable to right himself.

B. Absence of the normal reflex in a chronically treated animal.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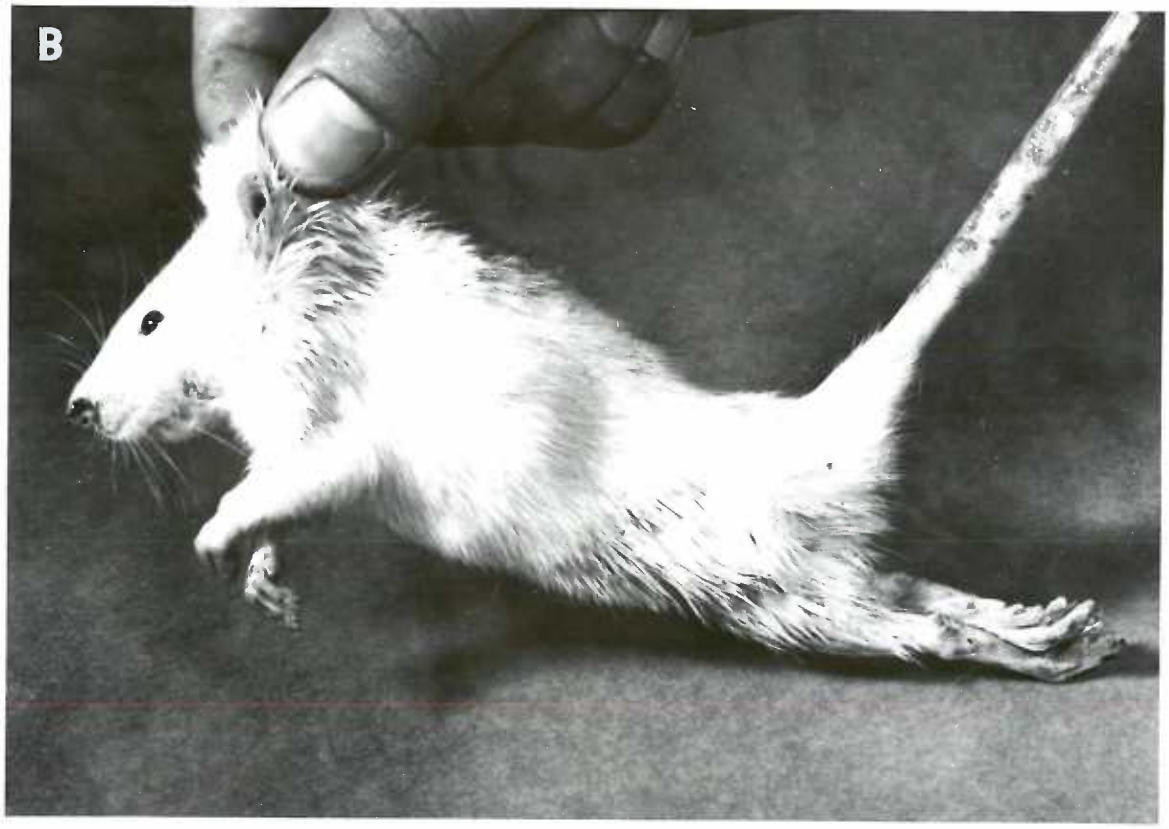

B

TABLE XIII THE EFFECT OF CHRONIC TRIETHYL TIN ACETATE INTAKE ON TOTAL BODY WEIGHT OF RATS.

STUDY 1

Days on Drug	TETA Treated (10 rats) Means	Pair-Fed Controls (7 rats) Means	Ad Lib Controls (7 rats) Means
0	145.9	148.4	150.0
2	135.8	135.4	156.1
4	136.8	136.0	159.9
6	129.1	148.9	181.9
8	117.2	145.7	196.9
10	106.3	136.0	212.1
12	105.8	141.4	229.0
14	99.8	154.9	237.1

STUDY 2

	(20 rats)	(6 rats	(5 rats)
0	146.1	144.5	149.8
3	144.5	163.3	180.4
8	124.8	145.7	205.8
12	99.7		
13	103.1	135.0	231.0
14	101.5		

FIGURE 10

THE EFFECT OF CHRONIC TETA INTAKE

ON BODY WEIGHT OF RATS

(STUDY I) -(TABLE XIII)

The body weight of the experimental animals has been plotted against the number of days on TETA. This is contrasted to similar tracings of non-treated pair-fed animals and of ad lib controls. In each case the number (N) of animals included in the various categories is indicated. The onset of muscular weakness as well as the time when animals began to die has been chronologically marked with arrows.

EFFECT OF CHRONIC TETA* INTAKE ON BODY WEIGHT OF RATS (I)

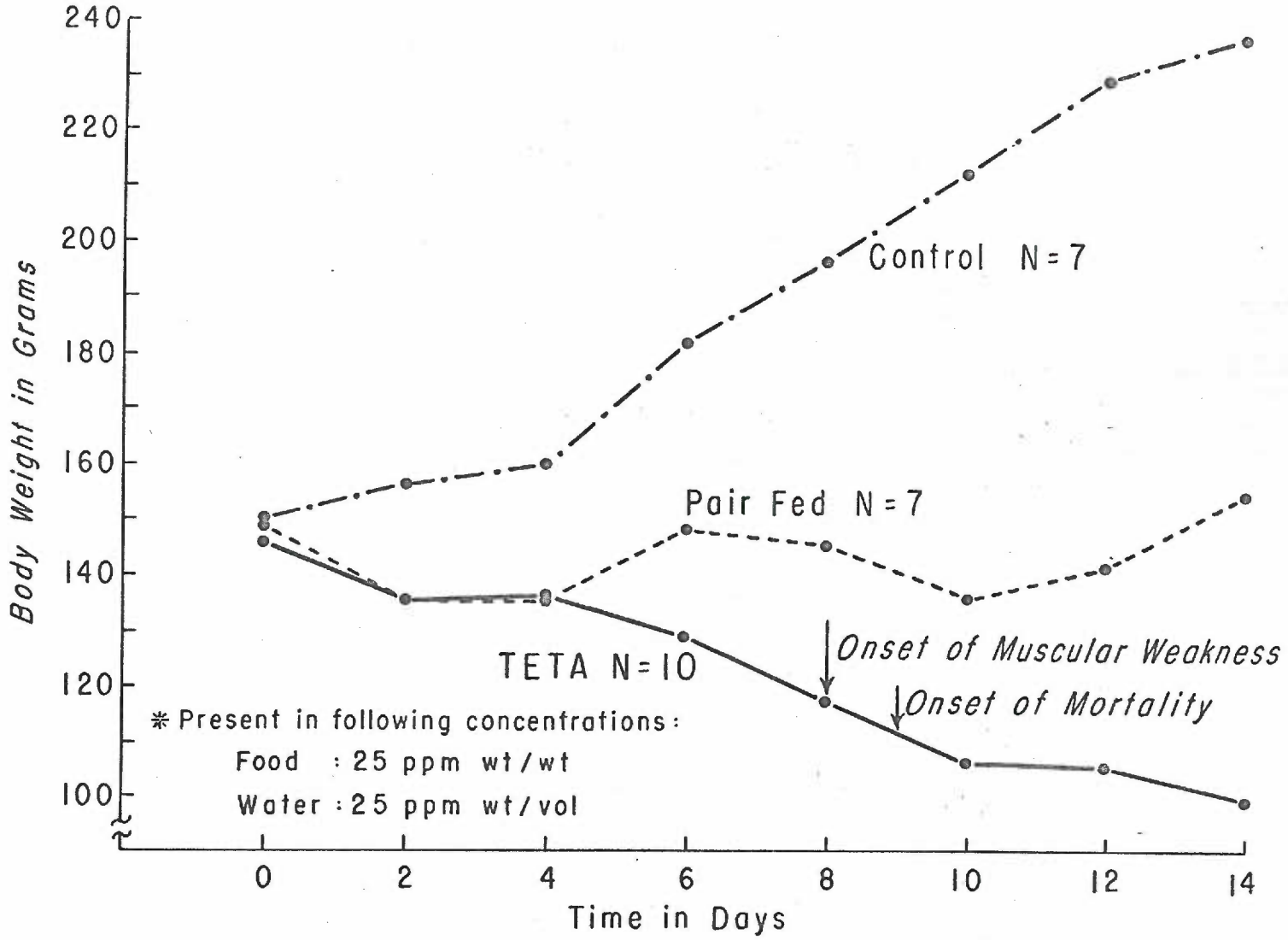


FIGURE 11

THE EFFECT OF CHRONIC BETA INTAKE

ON BODY WEIGHT OF RATS

(STUDY II) - (TABLE XIII)

This graph is similar to that shown in Figure 10 (see legend) representing the second study done on a different rat population.

EFFECT OF CHRONIC TETA* INTAKE ON BODY WEIGHT OF RATS (II)

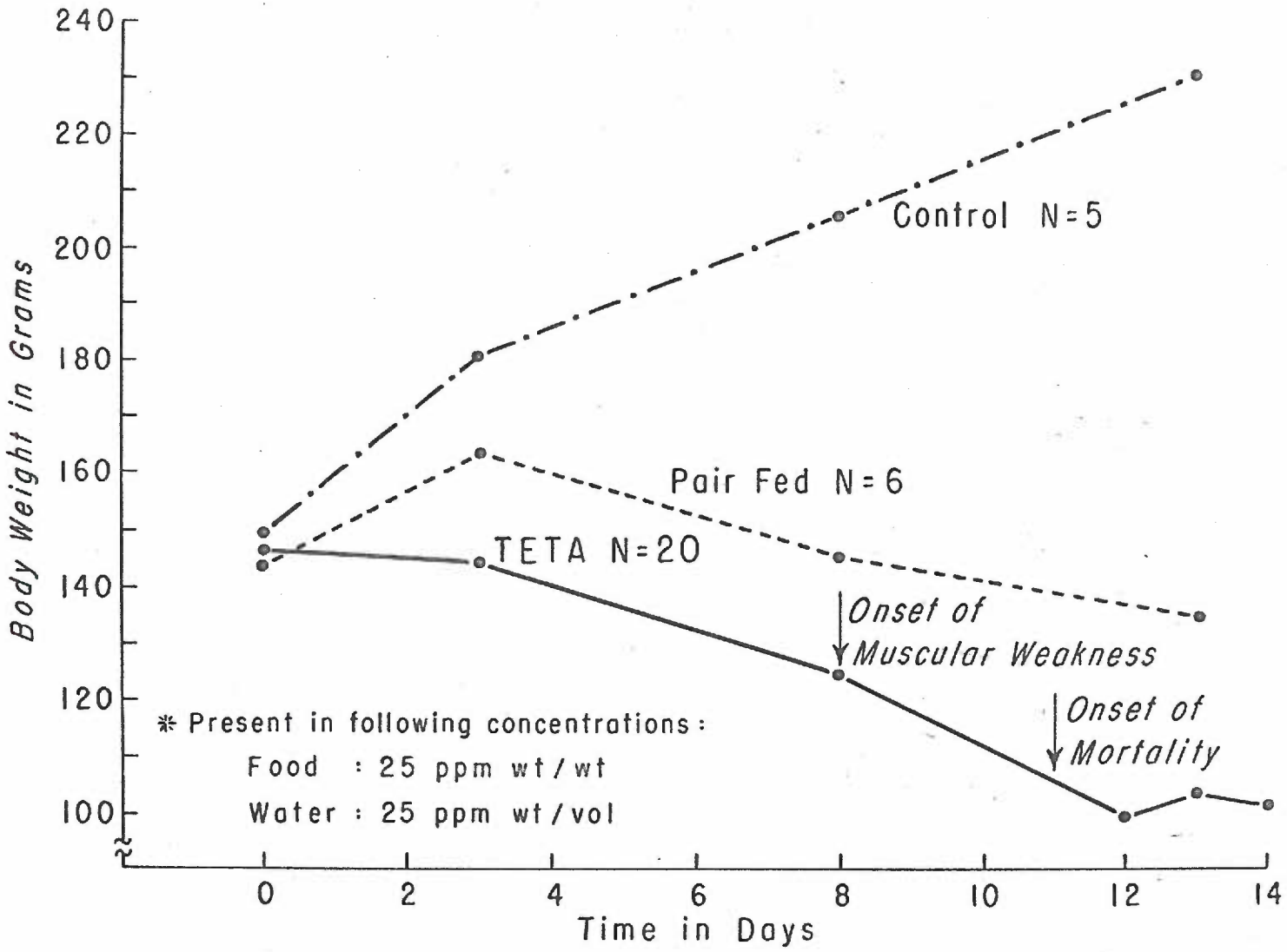
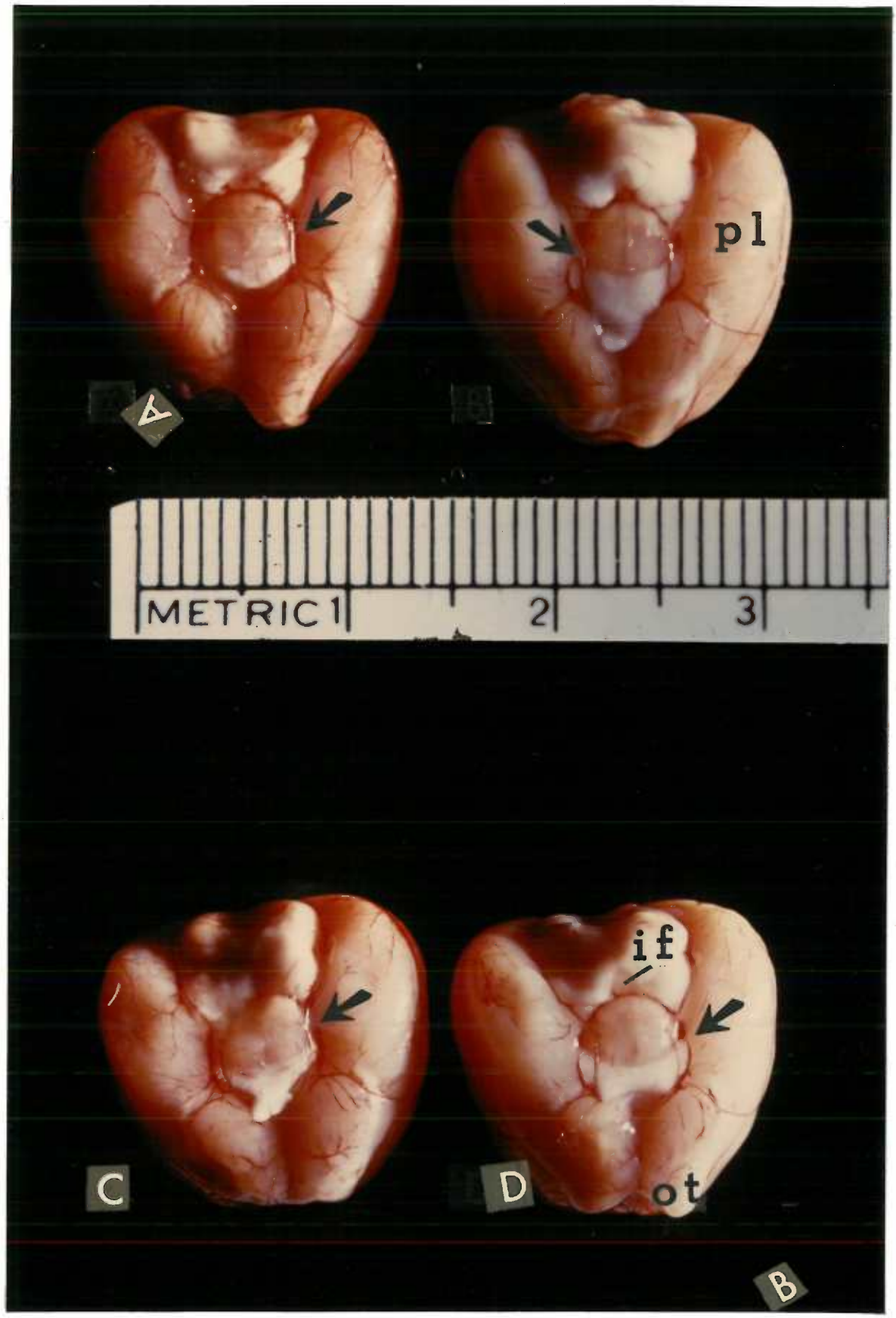


FIGURE 12

GROSS ANATOMICAL FEATURES OF BRAINS FROM CHRONICALLY TETA TREATED RATS (VENTRAL VIEW)

Normal brains from control rats (A and C) have been laid side by side to brains from rats treated with TETA for 14 days (B and D). The brains are all oriented in the same fashion so that the olfactory tracts are seen inferiorly (one of which is indicated as ot) while the interpeduncular fossa is shown superiorly (if) in the same specimen. The arrows indicate the space between the pyriform lobes (pl) (laterally) and the optic chiasm, tuber cinereum and mamillary body medially. The spaces are well outlined in the control brains while they are much reduced in the edematous brains. This has apparently resulted in a displacement (medially) or impingement of the internal carotid and posterior communicating arteries.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C. Effect of TETA on the Water Content of Rat Brain

1. Acutely treated animals (Figures 13, 14 and Table 14).

The brains from 27 animals injected 48 hours previously with TETA (10 mg/kg BW) were compared to the brains of 19 other animals used as controls. Besides having matched all these animals for sex, type, strain and several other parameters, they were also matched for weight. Thus, in the case of the controls, their mean body weight was 207 grams while the mean body weight of the experimental group was 201 grams for the controls (values falling between 185 and 229 grams) and 51 grams for the experimental animals (with values between 181 and 232 grams).

The mean percentage of brain water content in the controls was 79.1 per cent (with a mean percentage of dehydrated tissue weight of 20.9 per cent) while the same value for the TETA treated animals was 81.4 per cent (with a mean percentage of dehydrated tissue weight of 18.6 per cent). These two means were significantly different from each other at the $p < 0.001$ level. Using 20.9 per cent for the mean of normal dehydrated tissue weight, as calculated by Elliot and Jasper's method (36), the experimental animals were found to have a mean percentage brain swelling of 11.1 per cent.

The greatest range of water content of one hemisphere compared with the opposite one was 0.2 per cent for both controls and experimental rats. The range of the mean per cent water contents of the hemispheres

for the controls when all members of a population were considered was 0.3 per cent (the values falling between 78.9 and 79.2 per cent), while the similar range for the treated animals was 1.7 (with values going from 80.7 through 82.4 per cent). The range for percentages of brain swelling in the TETA treated animals was 8.1 per cent (with values between 7.7 and 15.8 per cent).

In Table 14 statistical significance was evaluated by using the Small Sample Theory "t" test (161) for means of two independent groups, with the number of scores in the two groups not equal.

Number of scores in Group I = N_1 (N_1= 7)

Number of scores in Group II = N_2 (N_2 = 9)

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\Sigma X_1^2 - (\Sigma X_1)^2/N_1 + \Sigma X_2^2 - (\Sigma X_2)^2/N_2}{(N_1 + N_2 - 2)} \left[\frac{1}{N_1} + \frac{1}{N_2}\right]}}$$

where X_2 = 167.474

X_1 = 146.551

X_2^2 = 3,118.722

X_1^2 = 3,068.294

$\bar{X}_2$ = 18.608

$\bar{X}_1$ = 20.936

$= \frac{2.328}{0.2109}$ t = 11.038

df (degrees of freedom) = $N_1 + N_2 - 2 = 14$

with 14 df a p = .001 requires a value for t of 4.140 or greater. Since t in this case = 11.038, the experimental group II mean is significantly different from the controls (I) at the $p < 0.001$ level.

FIGURE 13

EFFECT OF ONE DOSE OF TRIETHYL TIN ACETATE

ON THE WATER CONTENT OF RAT BRAIN

The statistical significance was calculated using the Small Sample Theory "t" test (161).

(The discrepancy existing between the value for the mean per cent swelling in the TETA treated animals , listed here as 11.1, and that given in Table 14, recorded as 11.357 per cent, is due to different methods of calculation.)

EXPERIMENTALLY INDUCED CEREBRAL EDEMA

Effects of One Dose of Triethyl Tin Acetate on Rat Brain

OBSERVATIONS	CONTROLS	TETA*
Mean % Water Content	79.1	81.4**
Mean % Dehydrated Tissue Wt.	20.9	18.6
Mean % Swelling	0	11.1***
Number of Animals	19	27
Hours after Injection	48	48

* 10 mg/kg Body Wt.
** Significantly different from controls at $p<0.001$
*** Calculated on the basis of 20.9 % mean dehydrated tissue wt. control

FIGURE 14

EFFECT OF ONE DOSE OF TRIETHYL TIN ACETATE

ON THE WATER CONTENT OF RAT BRAIN

These values are an expansion of those presented in Figure 13.

(T-test refers to the Small Sample Theory test used to document (in this case) lack of significant difference in body weights between experimental and control rat populations.)

EXPERIMENTALLY INDUCED CEREBRAL EDEMA (cont.)

Effects of One Dose of Triethyl Tin Acetate on Rat Brain

OBSERVATIONS		CONTROLS	TETA
% Water Content	Range 1*	0.3 (78.9-79.2)	1.7 (80.7-82.4)
	Range 2**	0.2	0.2
% Swelling - Range		0	8.1 (7.7 - 15.8)
Body Wt. - gm	Mean	207	201 (t-test)
	Range	44 (185 - 229)	51 (181-232)

* Range between different animals

** Greatest range in any single animal

TABLE XIV TABULATED MEAN VALUES FOR PER CENT WEIGHT LOSS OF RAT BRAIN (OR MEAN PER CENT WATER CONTENT) AFTER 18 HOURS OF DEHYDRATION.

(The mean per cent of dehydrated brain tissue weight and its square used in statistical analysis are both included.)

CONTROL SERIES

Group Number	Mean Per Cent Brain-Water Content	Mean Per Cent Dehydrated Brain Tissue Weight (X_1)	X_1^2
C1	79.175	20.825	433.681
C2	78.947	21.053	443.229
C3	78.902	21.098	445.126
C4	79.202	20.798	432.557
C5	79.200	20.800	432.640
C6	78.896	21.104	445.379
C7	79.127	20.873	435.682
	= 553.449	X_1 = 146.551	X_1^2=3,068.294
	Mean = 79.064%	$\bar{X}_1$= 20.936%	

AFTER ONE DOSE OF TETA

Group Number	Mean Per Cent Brain-Water Content	Mean Per Cent Dehydrated Brain Tissue Weight (X_2)	X_2^2	Per Cent Brain Swelling
A1	82.371	17.629	310.782	15.796
A2	80.855	19.146	366.469	8.550
A3	81.523	18.478	341.436	11.741
A4	81.305	18.696	349.540	10.699
A5	81.561	18.439	339.997	14.076
A6	81.802	18.198	331.167	13.078
A7	80.832	19.168	367.412	8.445
A9	81.598	18.402	338.634	12.104
A10	80.682	19.318	373.185	7.728
	=732.529	X_2=167.474	X_2^2=3,118.722	=102.217
	Mean = 81.392%	$\bar{X}_2$= 18.608		$\bar{X}$= 11.357%

2. Chronically treated animals (Figure 15, Table 15).

The brains from 28 rats treated with TETA for a period of 10 to 14 days are compared to the brains of 28 pair-fed controls. These two rat populations had a mean body weight of 143 and 148 grams, respectively, with a range of 31 grams in each case (from 124 to 155 grams in the experimental, and from 129 to 160 grams in the controls).

The mean per cent brain-water content in the control population was 78.5 per cent, while in the TETA treated animals it was 81.2, the latter representing a 12.5 per cent swelling in the edematous brains. The mean per cent dehydrated brain tissue weight for the experimental rats was 18.8 per cent and for the controls, 21.5 per cent.

The greatest range in per cent brain-water content in any single animal (when opposite hemispheres were compared) was 0.2 per cent for the controls and 0.3 per cent for the chronically treated animals. The greatest range when all members of a population were taken into consideration was 0.5 per cent for the controls (with values between 78.2 and 78.7 per cent) and 1.0 per cent for the experimental group (with values between 80.8 and 81.8 per cent).

The range in brain swelling for all members of the experimental population was 4.9 per cent with values going from 10.6 per cent to a 15.5 per cent increase in brain bulk.

FIGURE 15

EFFECT OF CHRONIC TETA INTAKE

ON THE WATER CONTENT OF THE RAT BRAIN

Both mean and range values are given (Table 15).

EXPERIMENTALLY INDUCED CEREBRAL EDEMA

Effects of Chronic TETA Intake on Rat Brain

OBSERVATIONS	CONTROLS*	TETA**
Mean % Water Content	78.5	81.2
Mean % Dehydrated Tissue Wt	21.5	18.8
Mean % Swelling	0	12.5
Number of Animals	28	28
Days on TETA	0	10-14

* Pair - fed controls

** Present in following concentrations { Food 25 ppm wt/wt; Water 25 ppm wt/vol }

EXPERIMENTALLY INDUCED CEREBRAL EDEMA

Effects of Chronic TETA Intake on Rat Brain (cont.)

OBSERVATIONS		CONTROLS	TETA
% Water Content	Range*	0.5 (78.2 - 78.7)	1.0 (80.8-81.8)
	Range**	0.2	0.3
% Swelling	Range	0	4.9 (10.6-15.5)
Gm. Body Weight	Mean	148	143
	Range	31 (129-160)	31 (124-155)

* Range between different animals

** Greatest range in any single animal

TABLE XV TABULATED MEAN VALUES FOR PER CENT BRAIN-WATER CONTENT AFTER 18 HOURS OF DEHYDRATION.

RAT POPULATION CHRONICALLY TREATED WITH TETA

Group Number	Mean Per Cent Brain-Water Content	Mean Per Cent Dehydrated Brain Tissue Weight	Per Cent Brain Swelling
CH1	81.816	18.184	15.459
CH2	80.794	19.206	10.707
CH4	81.620	18.380	14.547
CH5	81.269	18.731	12.916
CH6	81.043	18.957	11.865
CH7	80.768	19.232	10.586
CH8	80.916	19.084	11.274
	Σ = 568.226	Σ = 131.774	Σ = 87.354
	$\bar{X}$ = 81.175%	$\bar{X}$ = 18.825%	$\bar{X}$ = 12.479%

PAIR-FED CONTROLS

Group Number	Mean Per Cent Brain-Water Content	Mean Per Cent Dehydrated Brain Tissue Weight
CH11	78.637	21.363
CH12	78.442	21.558
CH13	78.653	21.347
CH14	78.307	21.693
CH15	78.186	21.814
CH16	78.539	21.461
CH17	78.673	21.327
	Σ = 549.437	Σ = 150.563
	$\bar{X}$ = 78.491%	$\bar{X}$ = 21.509%

D. Effect of TETA on Cations of the Rat Brain

1. Acute treated animals (Table 16, Figures 16, 17).

Fifteen brains obtained from experimental animals and 11 brains from controls were chosen from their respective groups for measurement of cation content, as explained in the section on Methods and Materials. However, for purposes of statistical analysis values for nine rat groups are listed in the experimental series, and seven from the control population.

Among the control electrolytes studied, potassium was found to have the highest value, and calcium the lowest. The means, in mEq/kg of dry tissue, plus or minus one standard deviation were: for potassium 332 $\pm$ 3.46, for sodium 220 $\pm$ 2.92, and for calcium 8 $\pm$ 0.23. The experimental edema values expressed in the same units were 342 $\pm$ 4.96 for potassium, 292 $\pm$ 15.69 for sodium, and 11 $\pm$ 0.43 for calcium. In terms of percentage, the experimental edema figures represent an increase of three per cent over the controls for potassium (difference significant at the $p < 0.001$ level), an increase of 31 per cent for calcium, and an increase of 33 per cent for sodium.

When the per cent increase in tissue sodium (in the experimental animals) was plotted against the degree of cerebral swelling (also expressed in percentage), a product-moment correlation, (r), of 0.98 was found. This, of course, suggested a direct relationship between these two variables (Figure 17).

FIGURE 16

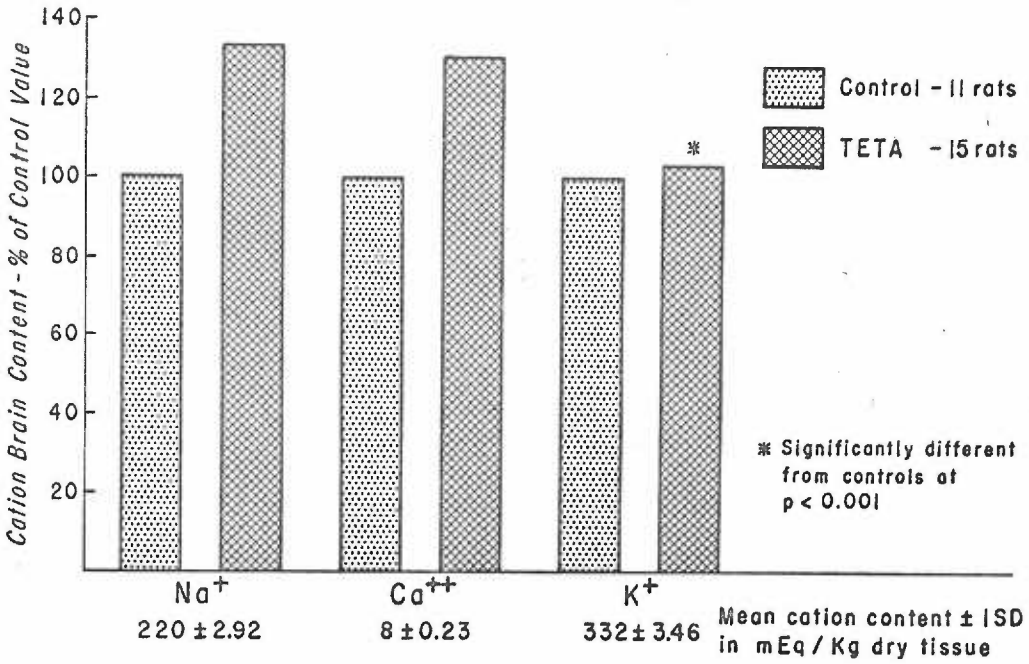
EXPERIMENTALLY INDUCED CEREBRAL EDEMA: EFFECTS

OF ONE DOSE OF TETA ON BRAIN CATIONS

In the upper part of this illustration the increase of cations (expressed in percentage) in the experimental edema animals is compared to the control values which have been arbitrarily set at 100 per cent. Significance was calculated by the small sample theory "t" test.

In the bottom part of the figure the direct mean values ± 1 standard deviation for both control and TETA-treated animals is given.

EXPERIMENTALLY INDUCED CEREBRAL EDEMA
Effects of One Dose of TETA on Brain Cations



EXPERIMENTALLY INDUCED CEREBRAL EDEMA
Effects of One Dose of TETA on Brain Cations

ELECTROLYTE VALUES IN mEq/Kg OF DRY TISSUE	CONTROLS	TETA
Na^+ - mean + ISD	220 ± 2.92	292 ± 15.69
K^+ - mean + ISD	332 ± 3.46	342 ± 4.96*
Ca^{++} - mean + ISD	8 ± 0.23	11 ± 0.43
Number of Rats	11	15

* Significantly different from controls at $p < 0.001$

TABLE XVI TABULATED MEAN VALUES FOR RAT BRAIN CATIONS: IN CONTROLS AND IN ANIMALS WHERE ACUTE CEREBRAL EDEMA WAS INDUCED BY THE INTRAPERITONEAL INJECTION OF ONE DOSE OF TETA

(I) EXPERIMENTAL ANIMALS $\bar{X}$ = Overall Mean

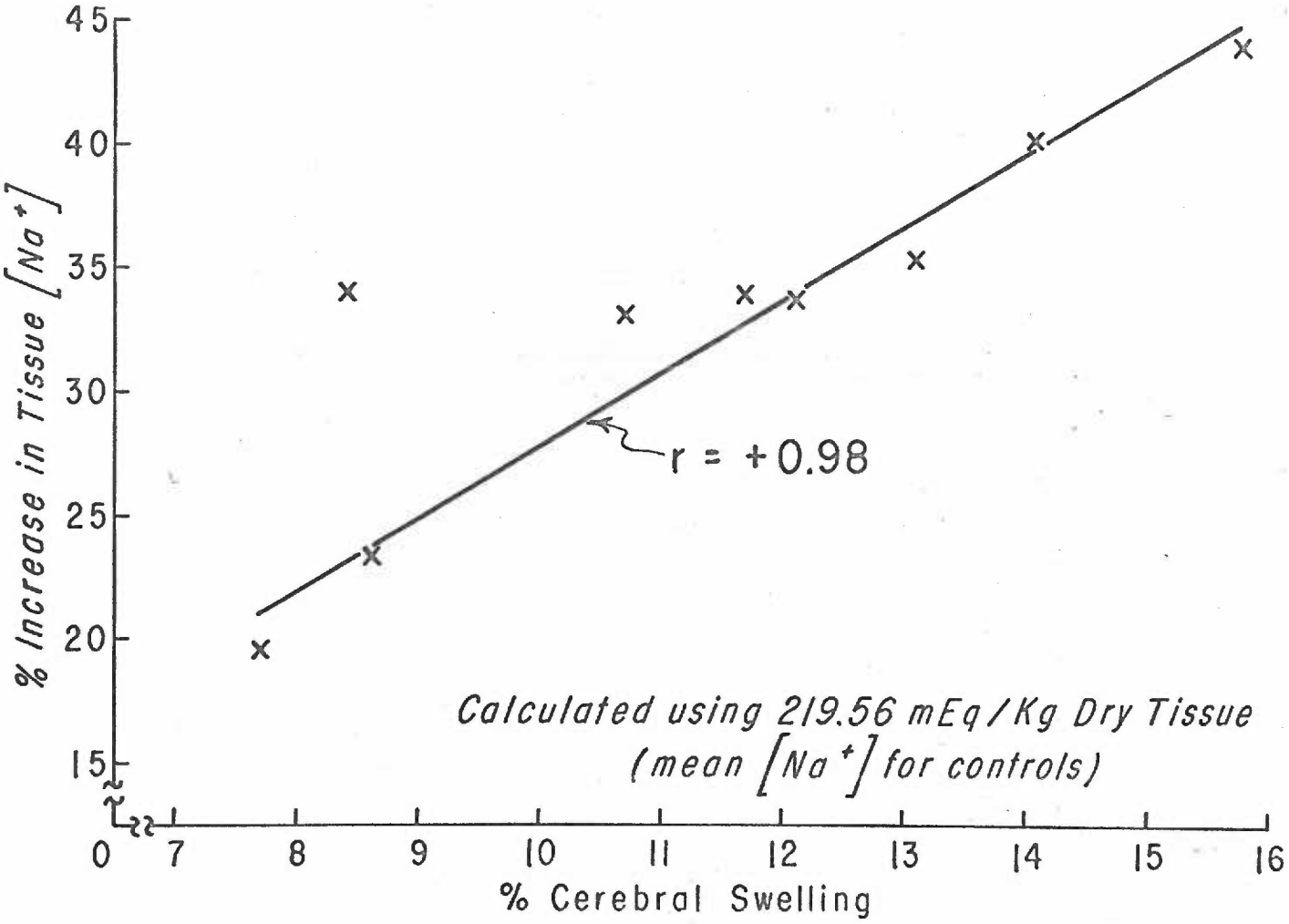
Group Number	Mean Brain Electrolyte Values (expressed in mEq/kg of dry tissue)		
	Sodium	Calcium	Potassium
A_1	317.065	11.133	338.695
A_2	271.140	10.090	335.554
A_3	294.145	10.915	341.777
A_4	292.505	10.460	345.959
A_5	308.015	10.960	346.952
A_6	297.435	10.560	342.497
A_7	294.290	10.460	350.920
A_9	293.700	10.760	336.054
A_{10}	262.770	9.700	338.344
	Σ = 2631.065	Σ = 95.038	Σ = 3076.752
	$\bar{X}$ = 292.341	$\bar{X}$ = 10.560	$\bar{X}$ = 341.861
(II) CONTROLS			
C	219.795	8.261	334.242
C	216.071	8.315	328.446
C	222.761	8.346	329.553
C	221.217	8.215	332.795
C	214.707	7.837	334.675
C	219.456	7.841	325.850
C	222.881	7.830	335.931
	Σ = 1536.888	Σ = 56.644	Σ = 2321.492
	$\bar{X}$ = 219.555	$\bar{X}$ = 8.092	$\bar{X}$ = 331.642

FIGURE 17

EXPERIMENTALLY INDUCED CEREBRAL EDEMA: RELATIONSHIP BETWEEN THE PER CENT INCREASE IN BRAIN-TISSUE SODIUM VERSUS THE DEGREE OF "CEREBRAL SWELLING" FOR TETA-TREATED ANIMALS

The control mean value for sodium of 219.56 mEq/kg of dry tissue (set at 100 per cent) has been used to calculate the relative increase in the experimental animals. The percentage of cerebral swelling was calculated as shown before, using 20.9 per cent as the mean per cent of normal dehydrated tissue weight. The best-fitting straight line was found by regression analysis.

EXPERIMENTALLY INDUCED CEREBRAL EDEMA



2. Chronically treated animals (Table 17, Figure 18).

Nineteen brains from experimental animals and 17 brains derived from controls were selected from two separate studies as explained in the section on Methods and Materials. Therefore, for purposes of statistical analysis, 12 rat groups are analysed in the experimental edema series, and 10 in the control population.

As in the acute studies, potassium was found to have the highest control electrolyte value and calcium the lowest. The means in mEq/kg of dry tissue plus or minus one standard deviation were for potassium 333.6 $\pm$ 10.90, for sodium 234.6 $\pm$ 14.8, and for calcium 5.1 $\pm$ 1.60. The corresponding experimental edema values were 341.9 $\pm$ 17.29 for potassium, 318.3 $\pm$ 11.97 for sodium and 7.6 $\pm$ 1.95 for calcium. These last figures when expressed in terms of percentage of the control values, represent an increase of 49 per cent for calcium, an increase of 36 per cent for sodium, and a non-significant increase of 2.5 per cent for potassium.

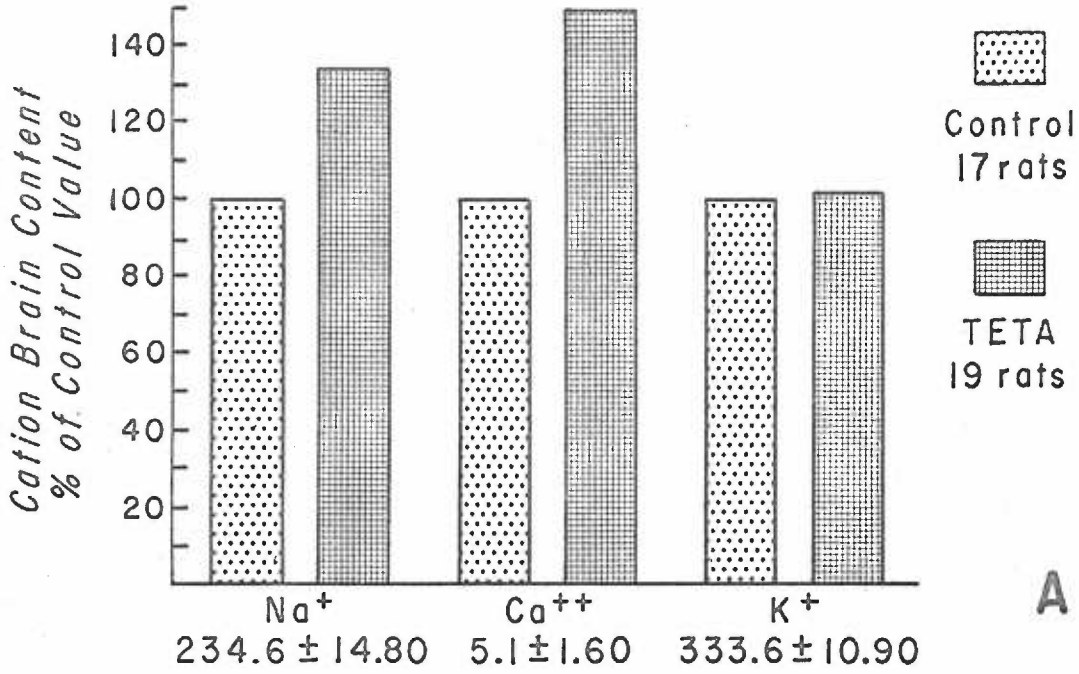
FIGURE 18

EXPERIMENTALLY INDUCED CEREBRAL EDEMA: EFFECTS OF CHRONIC TETA INTAKE OR BRAIN CATIONS

The upper part of this illustration shows the increase of cations (expressed in percentage) in the experimental animals when compared to the control values which have been arbitrarily set at 100%. Significance was calculated by the small sample theory "t" test.

The bottom part of the figure shows the direct mean readings ± 1 standard deviation for both control and TETA-treated animals.

EXPERIMENTALLY INDUCED CEREBRAL EDEMA
Effects of Chronic TETA Intake on Brain Cations



A

EXPERIMENTALLY INDUCED CEREBRAL EDEMA
Effects of Chronic TETA Intake on Brain Cations

ELECTROLYTE VALUES IN mEq/Kg OF DRY TISSUE	CONTROLS	TETA
Na^+ mean + 1SD	234.6 ± 14.80	318.3 ± 11.97
K^+ mean + 1SD	333.6 ± 10.90	341.9 ± 17.29**
Ca^{++} mean + 1SD	5.1 ± 1.60	7.6 ± 1.95*
Number of Rats	17	19

B

* Significantly different from controls at $p<0.01$

** No significant difference

TABLE XVII TABULATED MEAN VALUES FOR RAT BRAIN CATIONS: IN CONTROLS AND IN ANIMALS WHERE CEREBRAL EDEMA WAS INDUCED BY THE CHRONIC INGESTION OF TETA

(I) EXPERIMENTAL ANIMALS $\bar{X}$ = Overall Mean

Group Number	Mean Brain Electrolyte Values (expressed in mEq/kg of dry tissue)		
	Sodium	Calcium	Potassium
CH_1	331.272	8.961	323.331
CH_2	320.971	10.037	392.063
CH_4	311.289	8.985	352.104
CH_5	328.488	11.062	341.856
CH_6	321.881	7.130	342.507
CH_7	312.008	5.289	323.695
CH_8	333.795	3.662	328.330
CLH_3	327.280	7.742	343.742
CLH_4	326.717	6.511	335.201
CLH_{28}	291.757	7.479	343.144
CLH 29	307.485	6.339	334.525
CLH 26	307.144	7.927	341.888
	Σ = 3820.027	Σ = 91.124	Σ = 4102.386
	$\bar{X}$ = 318.336	$\bar{X}$ = 7.594	$\bar{X}$ = 341.866
(II) CONTROLS			
CH_{11}	252.632	3.518	346.131
CH_{12}	252.856	6.350	326.513
CH_{13}	260.925	6.760	345.399
CH_{14}	237.526	5.119	319.767
CH_{15}	222.693	5.014	324.115
CH_{16}	233.092	4.372	325.791
CH_{17}	223.194	2.691	332.601
CH_{21}	223.186	4.427	337.626
PL_{13}	219.599	8.450	353.438
PL_{16}	220.418	4.146	324.169
	Σ = 2346.121	Σ = 50.847	Σ = 3335.55
	$\bar{X}$ = 235.612	$\bar{X}$ = 5.085	$\bar{X}$ = 333.555

E. Light Microscopy of Rat Brain Tissue (Figures 19 through 24)

All of the rat tissues studied microscopically belong to animals which were fed TETA in their food and water for 10 to 14 days and the control animals for this experiment. Human tissue is shown in Figure 19 in order to illustrate a classical type of neuroglial reaction to noxious stimuli. The areas primarily studied in rat brain were the cortical and subcortical regions since these were found to best demonstrate the various types of histological aberration associated with triethyl tin edema.

The main findings noted in edematous brain tissue by light microscopy were found to agree with the observations made on the same type of tissue using the electron microscope. These are illustrated in Figure 20, Plates D, E and F where myelin sheath distention with vacuole formation is clearly visible. These vacuoles, containing a particle-free fluid, gave no stain reaction. In most cases it was possible to observe the demarcation of these vacuoles, or spaces, by myelin lamellae, eliminating the possibility that these were intercellular spaces. In several tissues these vacuoles had become confluent with each other due to disintegration or gap formation of their enclosing myelin lamellae.

At low magnification, these spaces gave the impression of just being gaps in the tissue without any particular demarcation, yet inspection at higher magnification usually revealed the true features presented above (Figures 21D, 22D and 22E).

Despite the fact that most of the reticulation occurred in the myelinated white matter, including areas of the corpus callosum, a few such spaces were found in the cortex proper (Figure 22, Plate D).

The other findings are related to various aspects of possible neuroglial response to noxious stimuli. Use of the PTAH (Mallory's phosphotungstic acid hematoxylin) method was decided because of its ability to stain pathological astrocytes better than normal. This staining method allowed the following features to be observed in the experimental edema material. The neuroglial cells (which had a deep purple-blue tinge) appeared to be astrocytes on the basis of morphological criteria even though in some cases their perineuronal position might have suggested other types such as the oligodendroglia. Their normally unstained cell body had now acquired a pink-bluish coloration and was clearly eccentric in relation to its nucleus, suggesting astrocytic swelling as pointed out by Greenfield (56). The neuroglial processes which also had been smooth and regular in appearance in control material, had undergone twisting and had become quite irregular with some of the processes broken off or fragmented. Increase in neuroglial feltwork was also apparent in some instances (Figure 23, Plate F). In conclusion, the sponginess observed primarily in the myelinated areas, coupled with the various possible astrocytic aberrations just described, gave a picture quite characteristic for this type of edematous preparation.

FIGURE 19

LIGHT MICROSCOPY OF HUMAN BRAIN TISSUE:

ASTROCYTES

Tissues from normal human brain cortex (Plates A, B, C) are contrasted to specimens derived from a human cortical glial scar (Plates D, E, F). All tissues were stained by the Cajal's Gold Sublimate Method.

(NOTE: These illustrations are presented so that the reader might observe in classical material a well-known reaction of one type of neuroglial cell to noxious stimuli.)

Plate A. Typical astroglial density of normal cortex. Astrocytes are stained purple-black against the much lighter background (x 285).*

Plate B. The same tissue at higher magnification. The astrocytes and their processes (light purple-black) are better visualized (see arrow) (x 1225).

Plate C. Astrocytes communicating with the capillary (c) by their foot-plates; one of them is shown at the arrow. (x 2764).

Plate D. Proliferation of astroglial fibers - gliosis - and hypertrophy of the astrocytic cell bodies (x 190).

Plate E. Higher magnification of tissue shown in Plate D shows intricate neuroglial feltwork in glial scar to advantage (x 1225).

(In both Plate D and E the original stain color has been changed due to photographic distortion.)

Plate F. Another area of gliosis permitting better observation of cell body and nuclear hypertrophy and pyknosis (x 1225).

*The magnification listed includes both microscopic resolution and photographic enlargement.

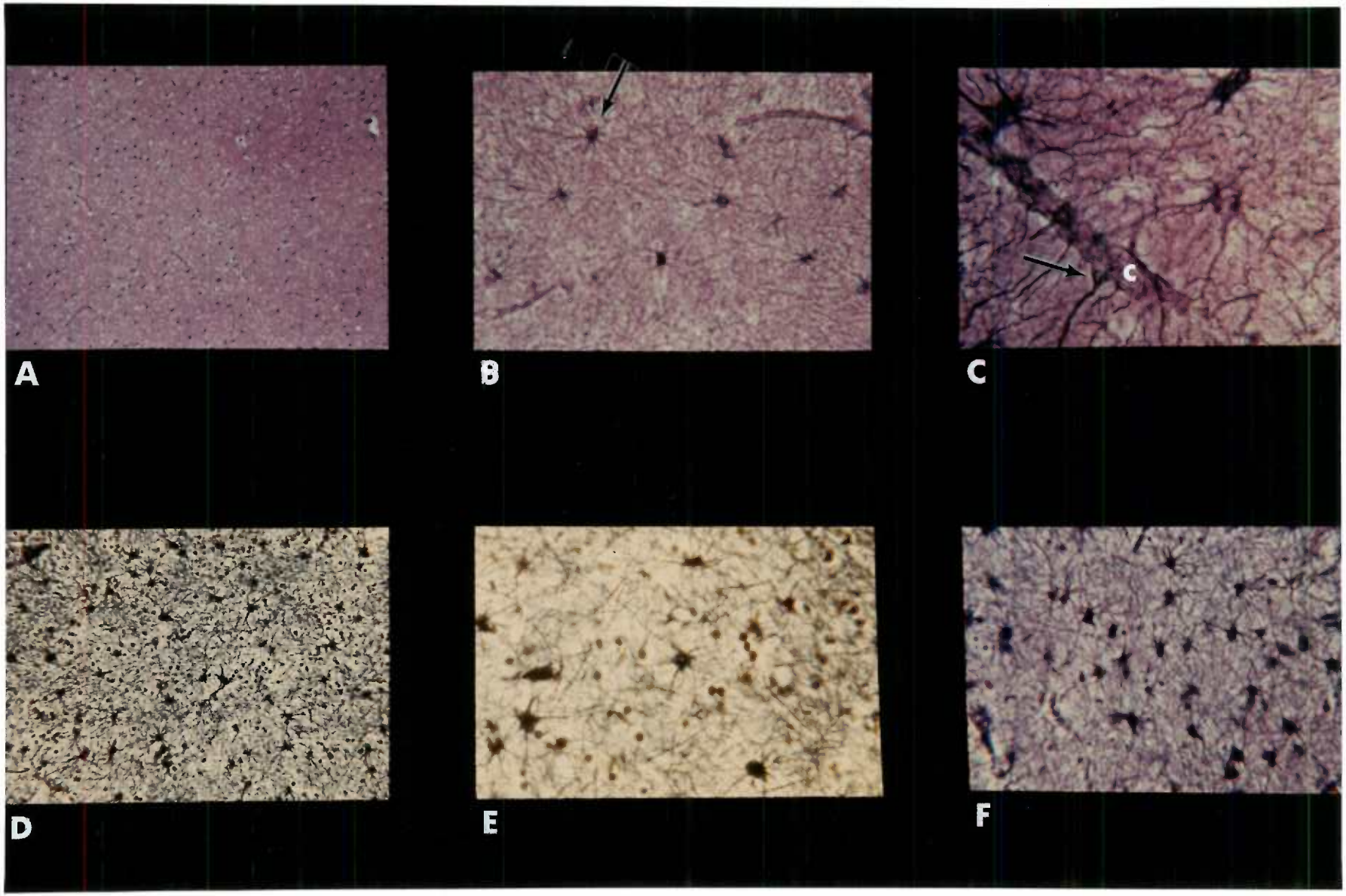

A
B
C
D
E
F

FIGURE 20

LIGHT MICROSCOPY OF RAT BRAIN TISSUE

Tissues were stained by the Azur II-Methylene Blue method. These are the "thick" sections (1 micron thick) of the blocks which were used for electron microscopy. Plates A, B, and C correspond to tissues from normal animals, while Plates D, E, and F are from tissues derived from rats where cerebral edema was induced by chronic TETA intoxication.

Plate A. Cellular density deep in the normal rat cerebral cortex, showing compactness of microscopic structures including neurons (n) and myelinated nerve fibers (see arrows) (x 1225).

Plate B. Same area at higher magnification. Notice neurone (n), astrocyte (a) and myelinated nerve fibers at the pointer (x 2764).

Plate C. Neurons with their myelinated processes deep in the cerebral cortex. Again, note compactness and cohesiveness of structures (x 2764).

Plate D. Area deep in the cerebral cortex somewhat comparable to area in Plate A showing relatively normal-appearing neurons (see pointer) and grossly distended myelin sheaths forming perfectly clear vacuoles (v). (x 1225).

Plate E. Magnification of section of Plate D showing contrast between compact cortex and reticulated appearance of immediately adjacent distended myelin sheaths. Note vacuoles (v) (x 2764).

Plate F. Further illustration of reticulated spaces or vacuoles (v) containing water-clear material. Some of the vacuoles have become confluent with each other by disintegration or development of gaps in their separating myelin lamellae (x 2764).

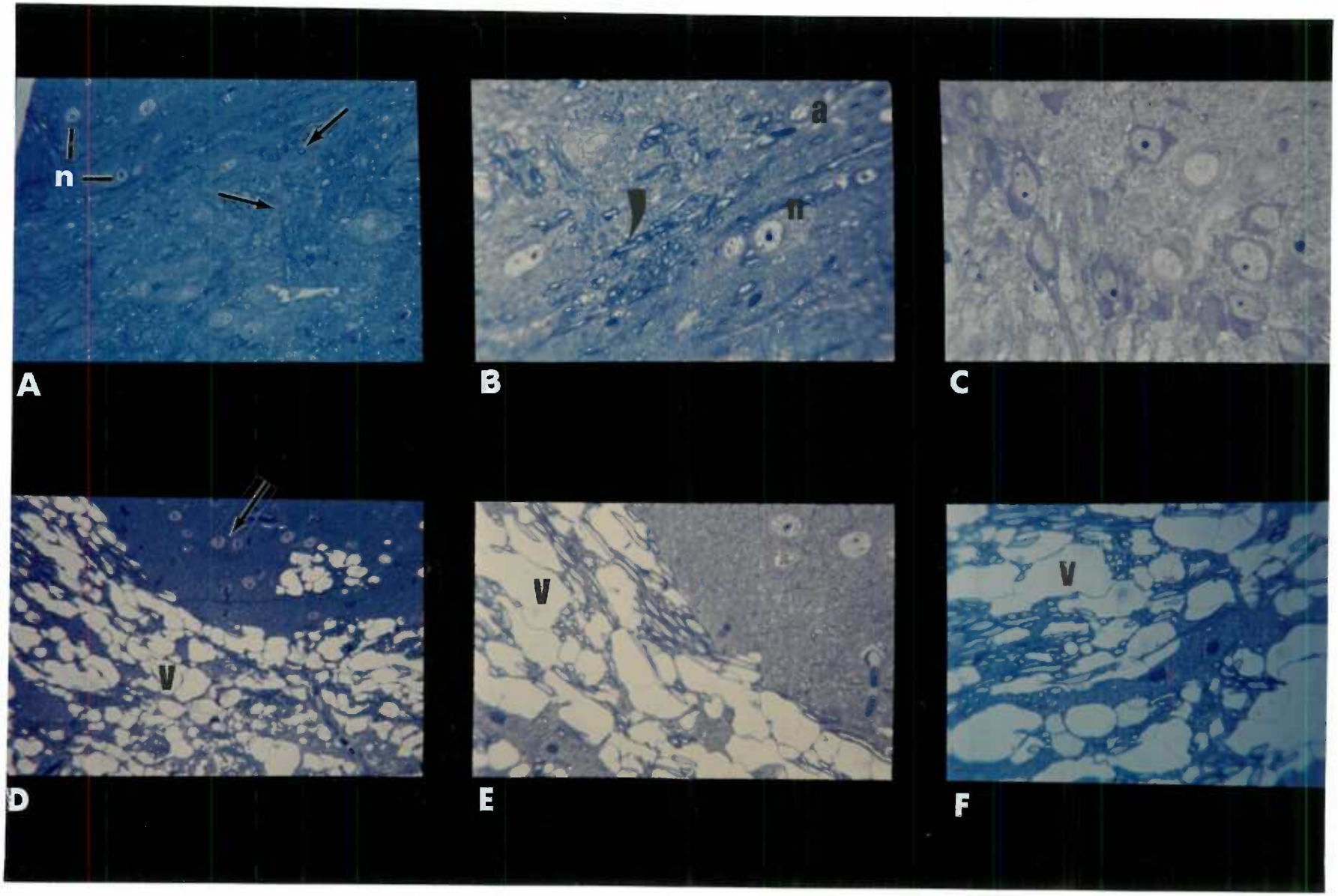

n
a
n
A
B
C
V
V
V
D
E
F

FIGURE 21

LIGHT MICROSCOPY OF RAT BRAIN TISSUE

Tissues were stained by Mallory's Phosphotungstic Acid Hematoxylin (PTAH) method. Plates A, B, and C correspond to tissues from normal animals, while Plates D, E and F are from tissues derived from rats where cerebral edema was induced by chronic TETA intoxication. The PTAH method gives nuclei and neuroglial fibers a blue tinge, and stains pathological astrocytes better than normal.

Plate A. Intact rat cerebrum showing the normal compact structure of the cerebral cortex (c), subcortical white matter (sw), and their areas' of "interphase" (see pointer) (x 143).

Plate B. Intact cerebrum again showing compact structure of the cerebral cortex (c), corpus callosum (cc), and the area of the nucleus caudatus/putamen (see pointer) (x 285).

Plate C. A different view of the same area as that shown in Plate B (x 285).

Plate D. This area corresponds roughly to the one shown for normal tissue in Plate A. Notice the compact appearance of the cerebral cortex contrasted to the spongy nature of the subcortical white matter (sw) (x 285).

Plate E. A different view of the same area as shown in Plate D, demonstrating more clearly the confluent nature of some of the reticulated spaces (see pointer) (x 285).

Plate F. Diffuse sponginess of myelinated areas. This tissue corresponds roughly to the one shown for normal tissue in Plate C. It is suggested that it represents sponginess (due to myelin sheath distention) of both subcortical white matter and corpus callosum. The tissue is oriented so that the cerebral cortex is immediately superior to the illustration (x 285).

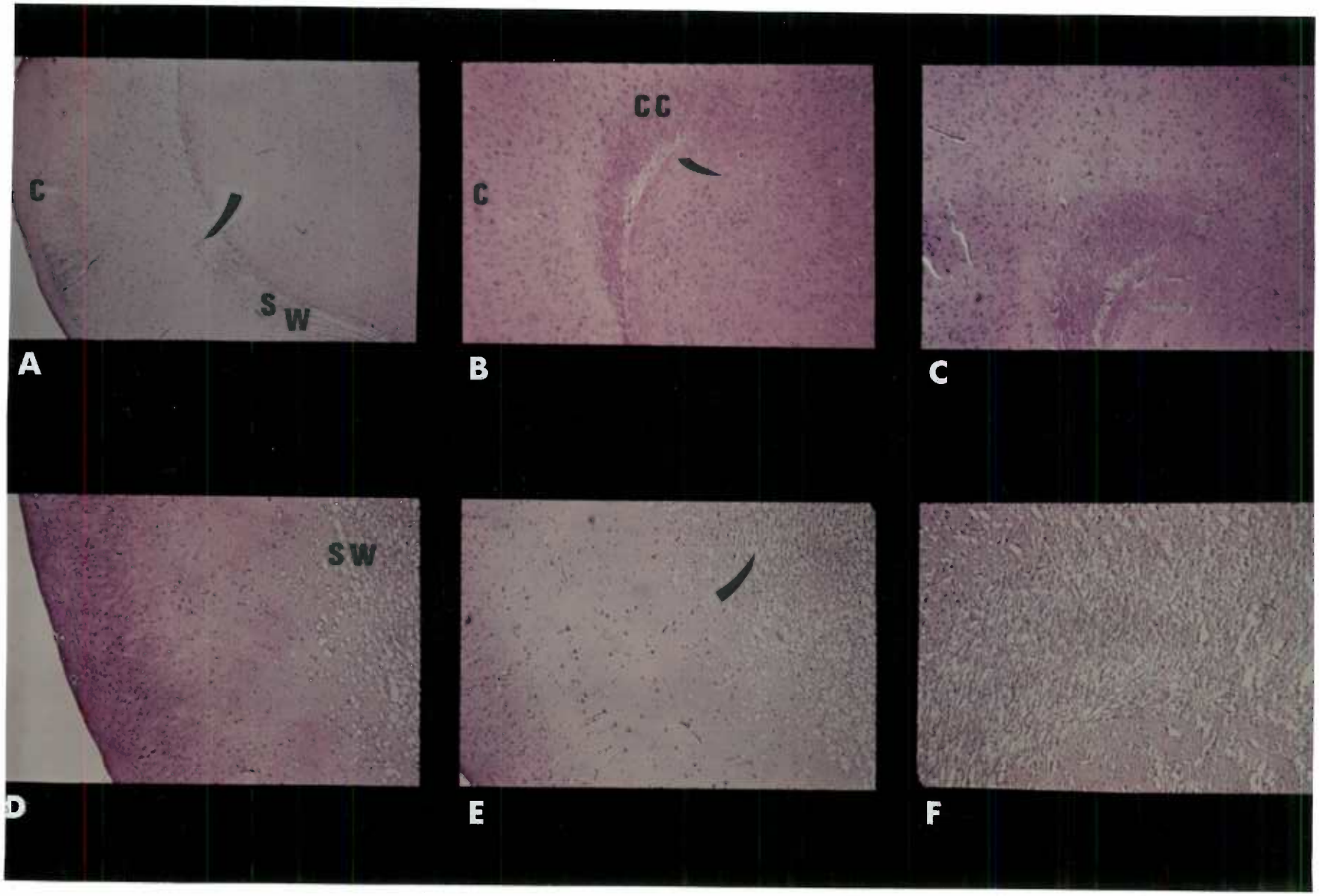

C
SW
A
CC
C
B
C
SW
D
E
F

FIGURE 22

LIGHT MICROSCOPY OF RAT BRAIN TISSUE

All of these brain tissues originated in rats in which cerebral edema was induced by chronic TETA intoxication. Tissues in Plates A, D and E were stained by using Schiff's PAS (periodic acid leucofuchsin) technique, while those in Plates B, C and F were stained by Cajal's Gold Sublimate method.

(NOTE: Original stain colors have been changed partly due to photographic distortion.)

Plates A, B and C. All show various degrees of reticulation (see arrows) or vacuole formation due to myelin sheath distention. Note that (in these studies) it has been the subcortical white matter which has suffered the most marked pathological alterations. The cortical area (c) is indicated in each case. Magnifications are as follows:

A. x 285.
B. x 143.
C. x 285.

Plate D. Myelin sheath distention and vacuole formation extending deep into the cortex (see pointers) (x 285).

Plate E. Magnification of one of the reticulated areas shown in Plate D. Note that the vacuoles are lined by myelin lamellae (see pointer), when these are intact, and are not perivascular. No staining reaction is evident inside the well-demarcated clear spaces (x 1225).

Plate F. This is an attempt at the demonstration at high magnification of myelin sheath distention in its early stages. Grossly normal-appearing cortex is illustrated in the lower part of the plate (x 1225).

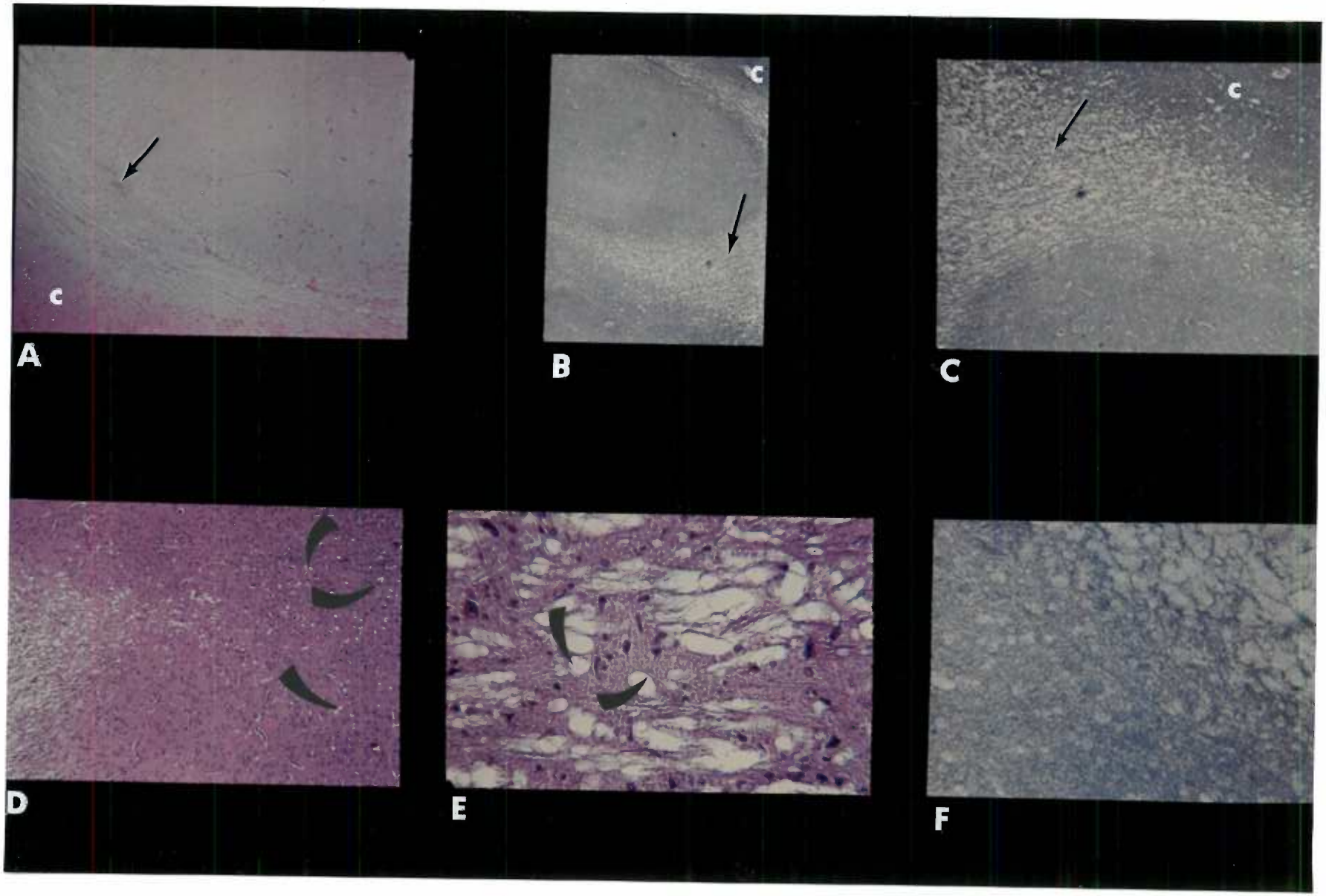

A
c
B
c
C
c
D
E
F

FIGURE 23

LIGHT MICROSCOPY OF RAT BRAIN TISSUE: ASTROCYTES

Tissues were stained by the PTAH method. Plates A, B and C correspond to tissues from normal brains, while Plates D, E and F are derived from rats where cerebral edema was induced by chronic TETA intoxication.

(NOTE: Although it is difficult to state with complete accuracy the identity of each of these neuroglial cells on the basis of the stain alone, suggestions will be made on the basis of this and other identifying features such as size of nucleus, shape and size of whole cell.)

Plates A, B and C are presented to illustrate density and staining properties of neuroglia deep in the cerebral cortex.

In A, B and C neurons (n) staining light pink are intermingled with the deep purple-blue tinged astrocytes (at end of pointers). Even though the perineuronal position of some neuroglia would suggest their being oligodendrocytes instead of astrocytes, this idea must be rejected because of the relative size of these cells when compared to the neurons. Note in B the relative regularity of the glial processes (see arrow). Also notice that the cell body is practically not visible (all one sees is the nucleus).

Plate D. Relative twisting and irregularity of the glial processes at the end of the arrows. Astrocytes (a) are indicated.

Plate E. Note the eccentric appearance of the cell body next to the nucleus (see pointers), suggesting relative swelling of the astrocytes.

Plate F. Conglomeration of astrocytes plus relative increase in neuroglial feltwork (see arrows). Many of the astrocytes have lost their processes.

That these are astrocytes and not other types of neuroglial cells is also indicated by the relative size and shape of the nucleus (as compared to the size of their cell bodies).

Magnifications for Plates A, B, C, D, E and F are as follows:

A. x 1225.
B. x 2764.
C. x 1225.
D. x 1225.
E. x 2764.
F. x 1225.

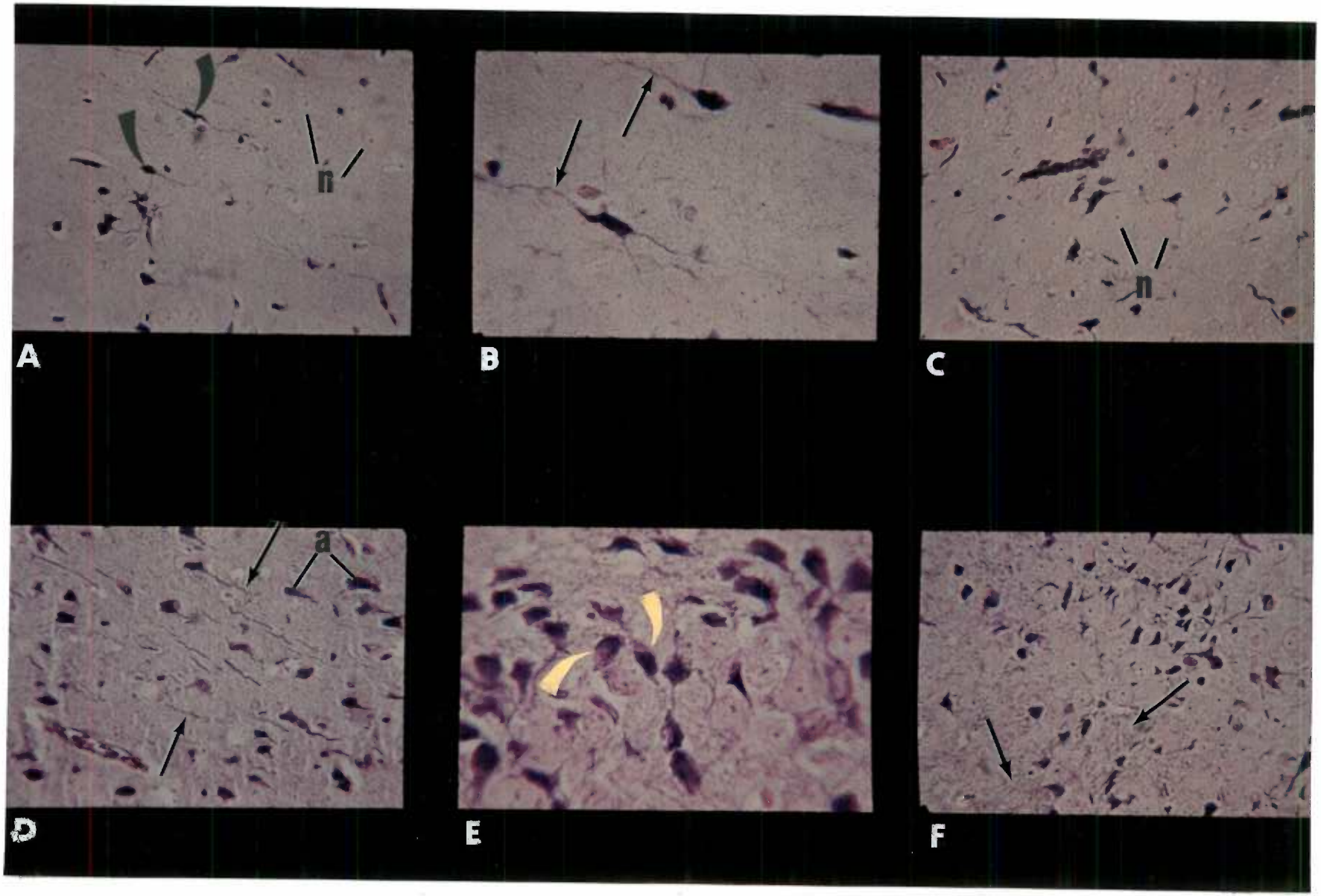

n
A
B
n
C
a
D
E
F

FIGURE 24

LIGHT MICROSCOPY OF RAT BRAIN TISSUE: ASTROCYTES

All tissues belong to animals where cerebral edema was induced by chronic TETA intoxication. Tissues were stained by the PTAH method.

Plate A. Typical vacuolar spaces give spongy appearance to subcortical white and cortical areas (x 285).

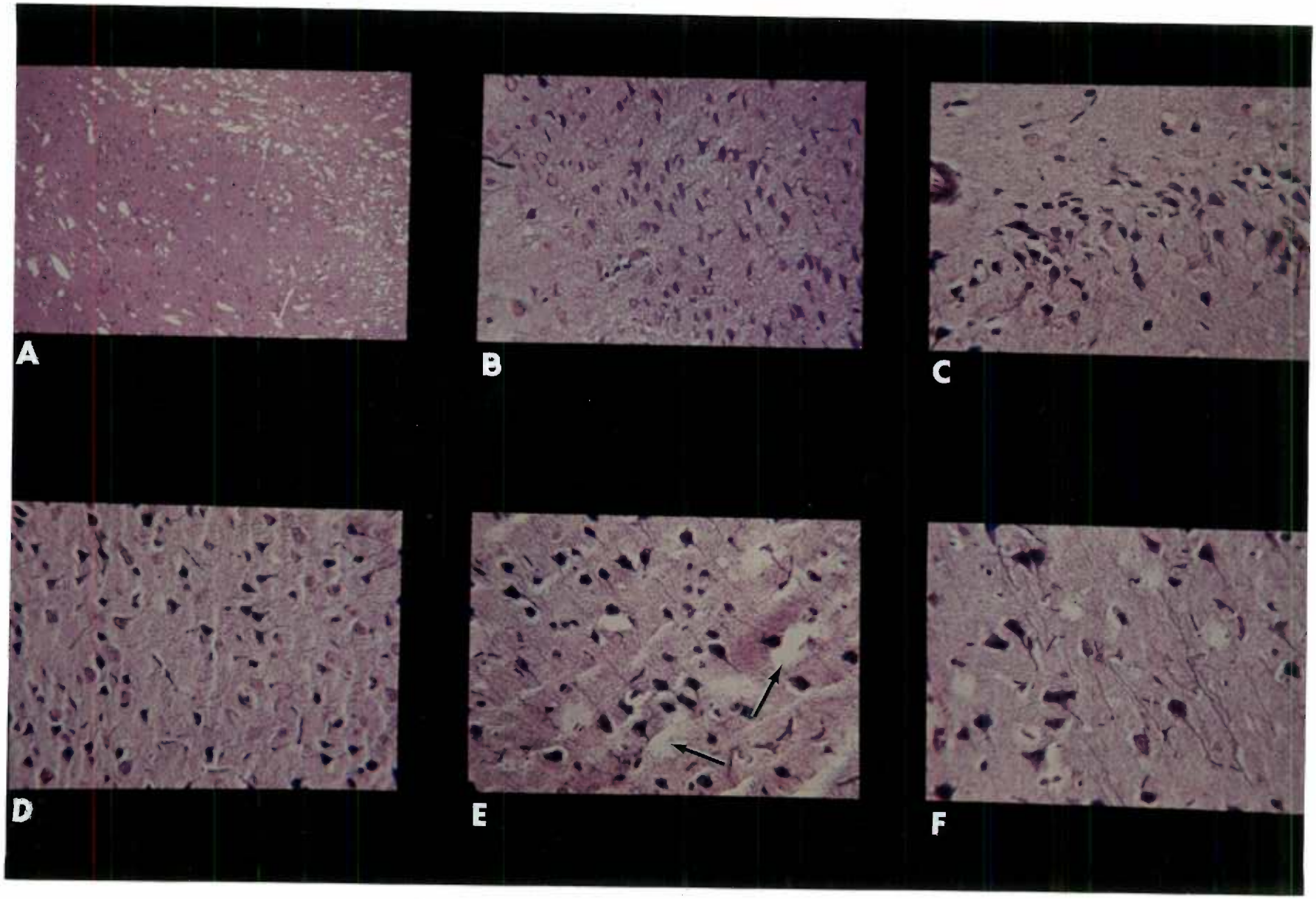
Plate B. A whole host of astrocytes without their processes suggesting some form of pathological transformation (x 1225).

Plate C. Further examples of perineuronal position by these neuroglial cells (x 1225).

Plates D, E and F are further examples of excess of glial fibers, irregularity and twisting of their processes and eccentric appearance of the astrocytic cell body. Clear spaces (at the end of the arrows) are artifacts in the tissue.

Magnifications for Plates D, E and F are as follows:

D. x 1225.
E. x 1225.
F. x 1225.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B
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F. Electron Microscopic Observations of Rat Brain Tissue

In identifying the various normal structures which one encounters in brain tissues, the criteria indicated by Schultz, Maynard and Pease (98, 140) and by Farquhar and Hartmann (38) have been adhered to when possible. This was necessary for the sake of consistency, in view of the current controversy and diverging opinions on this subject.

1. Observations in Normal Rat Brain

Illustrations of normal rat brain tissue are presented in Figures 25-27. Both glial and neuronal structures as well as their characteristic component elements are described in some detail. Emphasis has been placed in identifying the various features by which each cell is commonly recognized.

Besides the astrocytes, oligodendrocytes and microglia, a fourth type of cell, the "atypical", "intermediate" or "transitional" glial cell is presented. This cell classification therefore includes those cells which possess characteristics typical of more than one type of neuroglial cell.

Astrocytes are chiefly characterized by their size which, in many cases, is comparable to that of a neuron and usually larger than that of an oligodendrocyte; by their watery nucleus containing clumped chromatin masses; and by their cytoplasm which is also quite dilute and enclosed by an ill-defined cytoplasmic membrane. Overall, this cell has a "washed-out" or poorly preserved appearance. The astrocytic processes can be easily identified by their typical relation to blood

vessels and by their watery contents occasionally interspersed by mitochrondria or other cytoplasmic elements.

The oligodendrocytes are characterized by their smaller size, their moderately dense nucleus in which the chromatin is aggregated peripherally forming a dark rim along the nuclear membrane and by their moderately dense scant cytoplasm which also forms a narrow rim around the nucleus. Oligodendrocytes are situated typically between nerve processes ("interfascicular") or among neurons ("interneuronal").

The microglia are less common than both astroglia and oligodendroglia and are characterized by the extreme density of both their nucleus and cytoplasm. Also their cytoplasm seems to be more concentrated near one pole of the cell. The microglial processes when identified usually appear to branch angularly.

The neurons have a multitude of identifying features including a moderately dense nucleus containing granular material not particularly aggregated into chromatin clumps, a cytoplasm typically populated by RNA granules ("rosettes"), Golgi complexes, endoplasmic reticula and mitochondria. Neural filaments are also commonly seen at the site of the neuronal processes.

It is remarkable that except for the unidentified spaces mentioned in Figure 28, Plates A through D, no significant intercellular space was ever localized in either gray or white matter. On the contrary, there was always electron-dense material interspersed between identifiable structures.

The relation of astrocytic processes to vessels is demonstrated in Figure 28, Plates E and F. From inspection of the illustrations presented as well as from many others reviewed in the course of this study, it was evident that the astrocytic processes do make contact with the bare walls of small vessels, such as capillaries (which might be enclosed in their outermost aspect by a basement membrane) without the presence of a leptomeningeal sheath between these two entities. This sheath appears to surround vessels of larger size and apparently does not reach the capillary level.

2. Observations in TETA-Treated Animals (corresponding to Figures 29 through 31).

Similar findings were observed in tissues derived from animals where cerebral edema had been acutely induced as well as in those derived from animals where the cerebral edema had been produced over a period of 10 to 14 days.

The most important finding in this histological study was the very consistent and reliable presence of distended myelin sheaths forming vacuoles. Usually, but not always, these vacuoles were formed from the outer lamellae of the myelin sheaths. In some cases the distention was extreme so that the enclosing membrane would disintegrate or develop gaps in its surface. This could produce the erroneous impression of a large intercellular space.

At times the distended myelin sheaths would infringe upon adjacent structures deforming them or producing gaps in their surfaces, as is illustrated in Figure 30, Plates A, B, C and D.

Another finding without the significance of the one just mentioned, was the observation both in "acute" as well as in "chronic" material of apparent breaks in the plasma membranes of astrocytic processes. This fact was surprising in view of the finding of grossly normal structures (with the exception of mitochondria) surrounding the glial processes and also because the astrocytic processes did not seem particularly enlarged, at least not to the extent described by Torack, Terry and Zimmerman (164) in triethyl tin-treated mice. Similarly, the mitochondria found in the astrocytic processes were larger than their counterparts in normal tissue, as evidenced by direct measurement of the photomicrographs, taking into account their respective magnifications. Also their internal ridges or crests, when visualized, appeared to have lost their orderly arrangement. This finding could probably not be explainable by attributing it to variation in the plane of section.

Finally, no real extracellular space was ever observed despite the finding at times of enough histological evidence to substantiate the increased presence of fluid within the myelin sheaths.

Key for Figures 25 through 31

a - astrocyte

ac - astrocytic cytoplasm

ah - axon hillock

an - astrocytic nucleus

ap - astrocytic process

art - artifact

ax - axis cylinder (or axoplasm)

b - blurring

bm - basement membrane

en - endothelial nucleus

er - endoplasmic reticulum

gc - Golgi complex

l - lumen

m - mitochondria

mc - microglial cytoplasm

mn - microglial nucleus

mnf - myelinated nerve fiber

ms - myelin sheath

nc - neuronal cytoplasm

nf - neural filament

nm - nuclear membrane

nn - neuronal nucleus

np - nerve process

ncl - nucleolus

o - oligodendrocyte

oc - oligodendroglial cytoplasm

on - oligodendroglial nucleus

p - process

rbc - red blood cell

s - unidentified space

sc - smooth muscle

sm - synaptic membrane

ss - sarcolemmal sheath

sv - synaptic vesicle

unf - unmyelinated nerve fiber

v - vacuole in normal tissue

V - abnormal finding (vacuole) in experimental material

wbc - white blood cell

FIGURE 25

ELECTRON MICROSCOPY OF NORMAL RAT BRAIN: NEUROGLIA

Plate A. Astrocyte with watery cytoplasm ac and nucleus an containing characteristic clumped chromatin masses. Notice irregularly oval shape of nucleus together with the relative empty character of their poorly demarcated cytoplasm. All of these characteristics contribute to make this cell appear poorly preserved, which is one of its chief identifying features (7,281).

Plate B. Astrocytic process ap with typical watery appearance and occasional mitochondria m. Also notice the low density vacuoles which could be part of a Golgi complex and the relative sparsity of any other elements or particles. The arrow points to the tenuous membrane separating the process from the surrounding neuropile which contains abundant mitochondria and at least two small myelinated nerve fibers, mnf (10, 278).

Plate C. An oval, dense oligodendroglial nucleus on is seen, further characterized by its peripherally aggregated chromatin which forms a dark rim along the nuclear membrane. The scant rim of moderately dense cytoplasm is classical (x 5, 160).

Plate D. Another typical oligodendrial cell with nucleus on and cytoplasm oc which forms narrow rim and contains a moderate quantity of RNA particles (see arrow) and mitochondria. Its position among mitochondria-containing mg myelinated nerve fibers, mnf categorises it as an interfascicular oligodendrocyte (x 14,520).

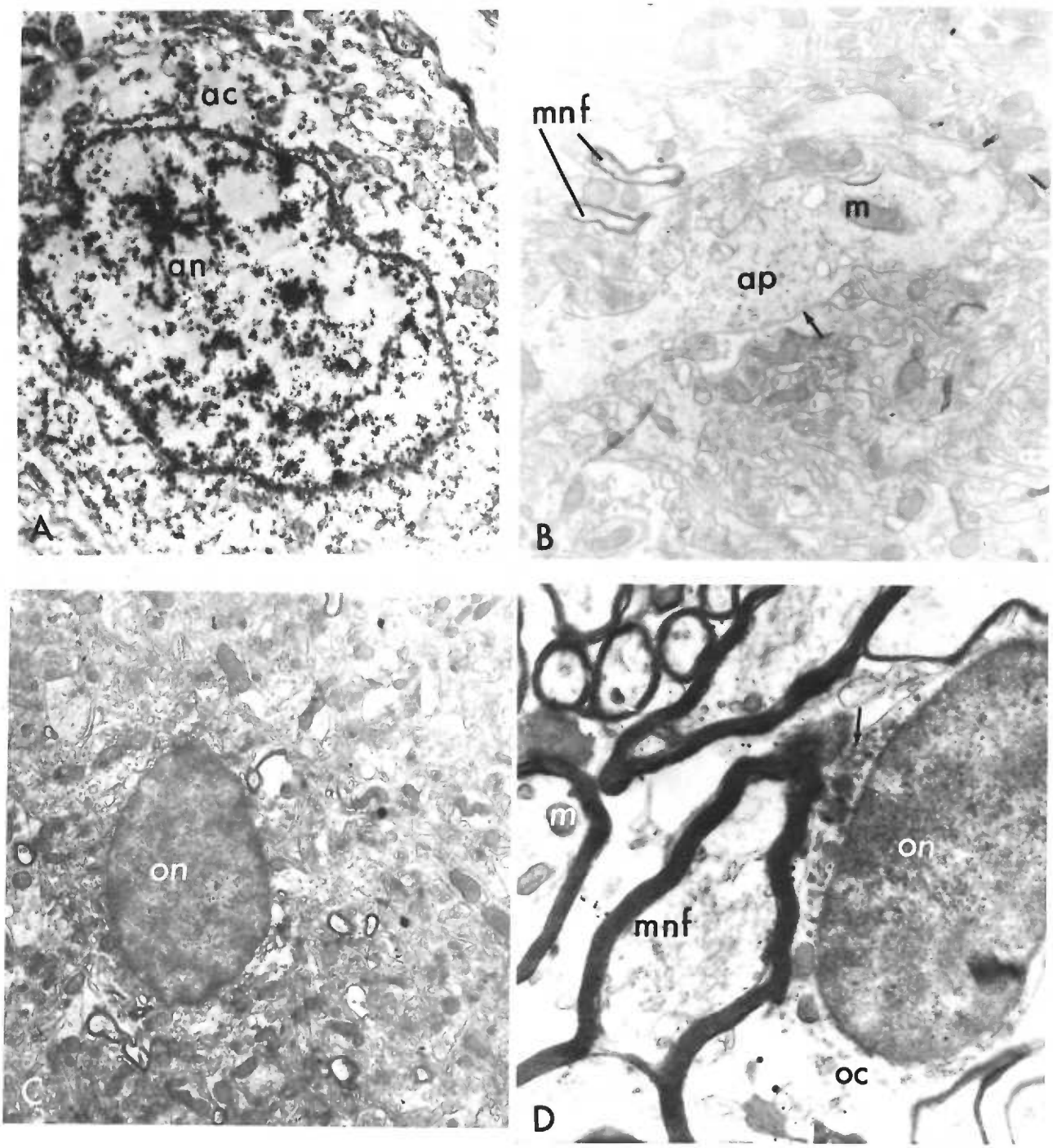

ac
an
A
mnf
m
ap
B
on
C
m
on
mnf
oc
D

FIGURE 26

ELECTRON MICROSCOPY OF NORMAL RAT BRAIN

Plate A. Myelinated nerve fibers cut in different planes, some of them containing an occasional mitochondrion, a few RNA particles and some neural filaments (x 14,502).

Plate B. Myelinated nerve fibers showing neural filaments nf, oriented in a fairly parallel direction; mitochondria m; and axoplasm ax. The myelin sheath ms has a lamellar structure, not too apparent in this illustration (x 20,462).

Plate C. This section is a magnification of the square demarcated in Figure D and shows myelinated nerve fibers, mnf, part of a microglial cell with extremely dense nucleus and cytoplasm, an astrocytic process ap with a mitochondrion m, and part of an oligodendroglial nucleus on. Note also the abundant synaptic vesicles sv, synaptic membranes (see arrows), the surprising relationship of the astrocytic process to the adjacent nerve processes and the close approximation of all structures resulting in a negligible intercellular space (x 20,462).

Plate D. Typical microglial cell characterized by its extreme overall density which makes differentiation between nucleus nn, and cytoplasm nc fairly difficult. The microglial cell is surrounded by myelinated and unmyelinated nerve fibers and bears close proximity to an astrocytic process and an oligodendrocytic whose nucleus on is shown (Figure C). The microglial process p retains the same heavy material density typical of both nucleus and cytoplasm. An extremely fine nerve process may be seen at the arrow (x 5,160).

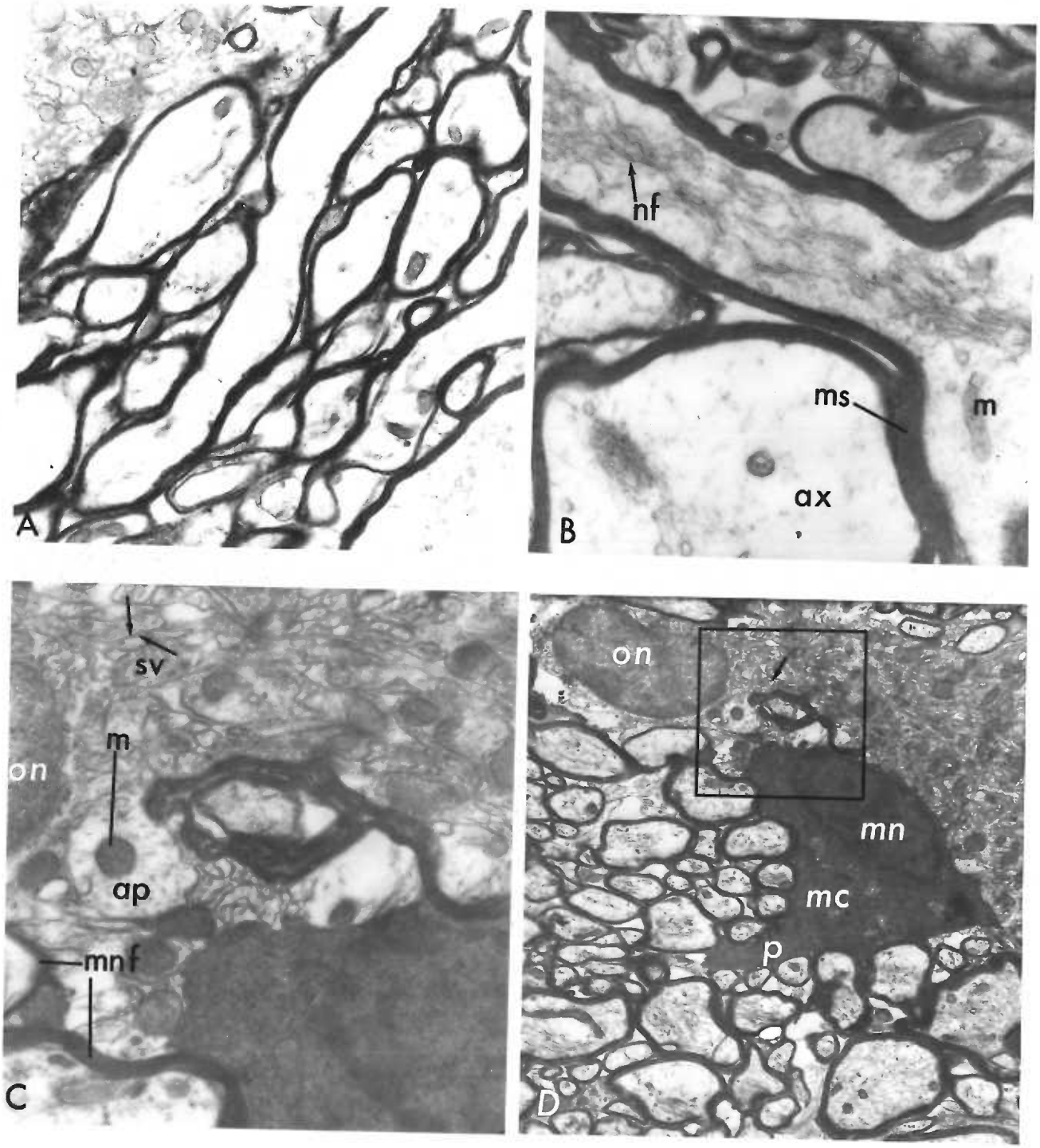

A
nf
ms
m
ax
B
sv
m
on
ap
mnf
C
on
mn
mc
p
D

FIGURE 27

ELECTRON MICROSCOPY OF NORMAL RAT BRAIN

Plate A. Neuronal nucleus nn and cytoplasm nc lying next to myelinated nerve fibers. The nucleus is enclosed within a double nuclear membrane nm and is constituted by granular material not particularly aggregated into chromatin clumps. The cytoplasm contains groups of dense particles (RNA granules) grouped as rosettes visible at the arrow, mitochondria, a vacuole v, and an aggregation of structures probably representing membranes, cannaliculi and vesicles of a Golgi Complex gc (x 20,462).

Plate B. Magnification of a section of adjacent Figure A. The double nuclear membrane is better visualized. The Golgi membranes supposedly have no attraction for RNA particles and thus differ from the membranes of endoplasmic reticulum in this very important point. Another feature of the endoplasmic reticulum is their tendency toward random orientation. The vacuole in the neuronal cytoplasm and the mitochondria are further demonstrated. The lack of demonstrable internal ridges or crests inside the mitochondria does not rule them out since it is well known that the plane of section must be nearly perpendicular to any membrane for it to be properly identified (x 57,482).

Plate C. Astrocyte exemplifying conspicuous chromatin clumping (at the arrows) and watery appearance of the remaining nucleoplasm an. The nucleolus ncl is probably visible as indicated. The cytoplasm ac is poorly demarcated and indented at least by two astrocytic processes ap as shown, one of which bears mitochondria (x 7,281).

Plates D and E. These are thought to represent "atypical", "transitional" or "intermediate" forms of neuroglial cells. Both of these cells (D and E) have some features ordinarily ascribed to oligodendroglia and some features ordinarily ascribed to astrocytes. Their size would tend to place them in the "oligo" category since these are smaller than astrocytes. Their nucleus has both clumped chromatin and a remaining watery nucleoplasm (features of astrocytes) and dark chromatin rims along the nuclear membranes (typical of oligodendrocytes). Again their cytoplasm is poorly demarcated, especially in Plate D, and yet, it is not watery but full of cytoplasmic structures. Their location among groups of axons, in this case myelinated nerve fibers mnf, is a more common characteristic of oligodendrocytes. Notice in Plate E the neural filaments nf approaching the glial cell from the left and probably belonging to a different plane (x 7,281).

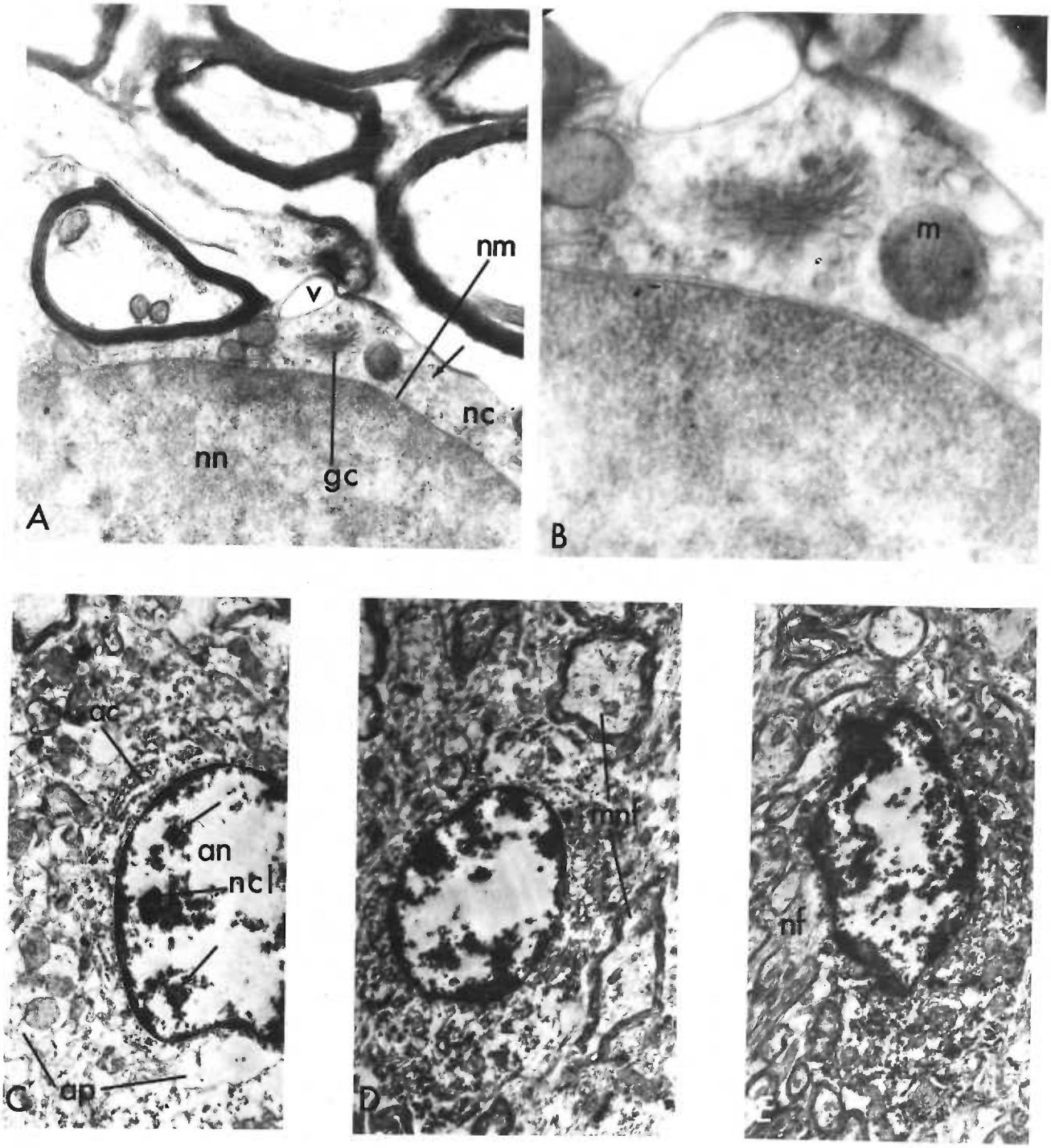

nm
v
nc
nn
gc
A
m
B
ac
an
nc
ap
C
mnf
D
nf
E

FIGURE 28

ELECTRON MICROSCOPY OF RAT BRAIN TISSUE

Plate A. Neuron with nucleus nn containing a good deal of granular material diffusely spread over the entire nucleoplasm and bounded by a double nuclear membrane. Solid black deposits in nucleus and cytoplasm art are holes in the tissue. The cytoplasm nc shows mitochondria m; typical rosettes of RNA granules at the arrows; endoplasmic reticulum er with RNA particles attached to its membranes; and component parts (vesicles and membranes) of a Golgi Complex gc with few or no attaching RNA particles. Note beginning neuronal processes at either end of the cell (x 10, 278).

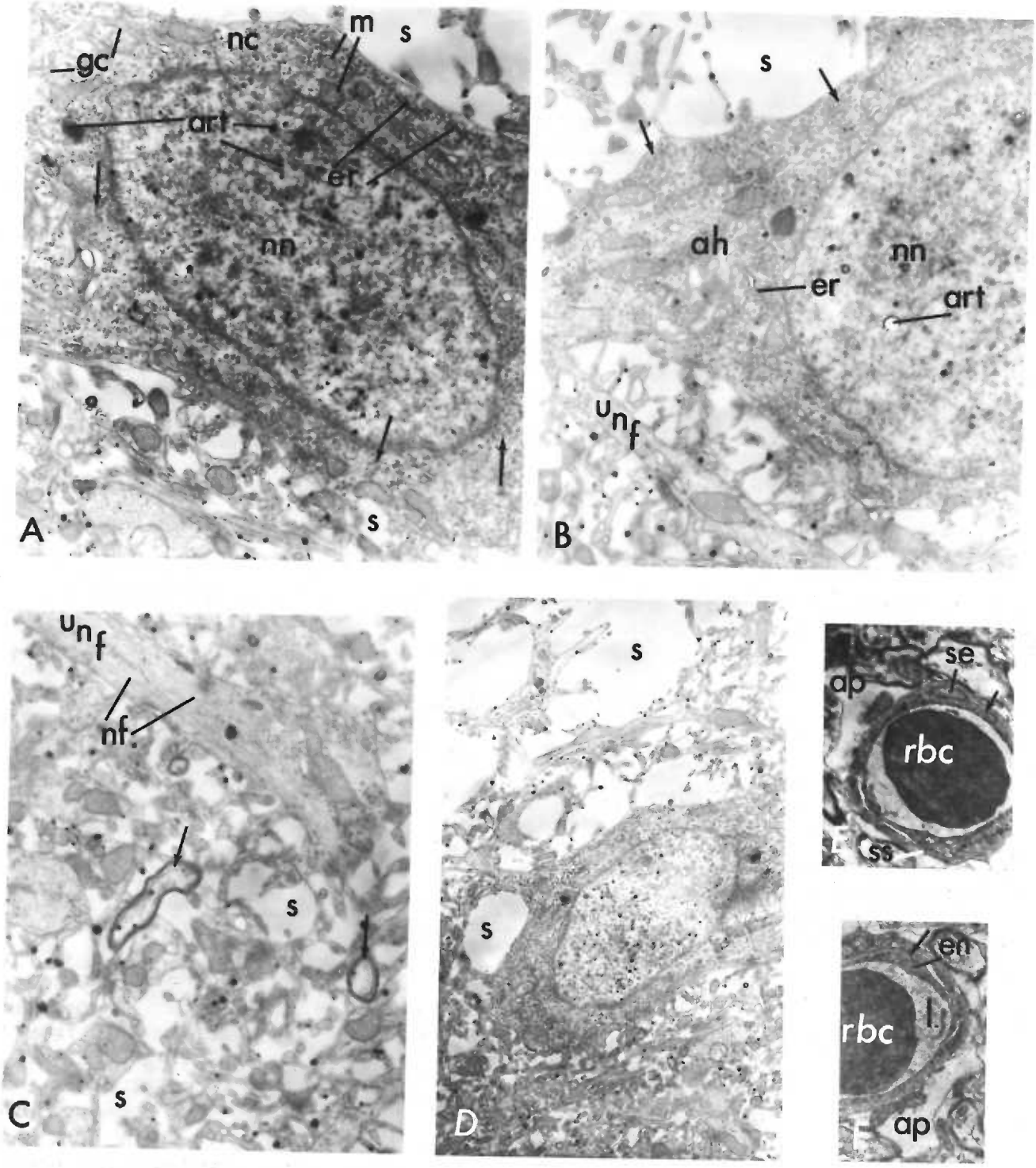
Plate B. Axon hillock region of neuron ah illustrating adjacent nucleus nn and containing mitochondria, endoplasmic reticulum er, vacuoles and rosettes of RNA granules at the arrows. The nucleus contains a carbon deposit art. An unmyelinated nerve fiber unf with a mitochondrion can be seen in the lower part of the field (x 10, 278).

Plate C. An unmyelinated nerve fiber unf with neural filaments nf is shown to advantage in upper part of plate. Small myelinated nerve fibers are also visible at the arrows (x 14,502).

Plate D. Neuron with same characteristics as those described for Plate A and partially surrounded by the unidentified spaces s mentioned below (x 5,160).

Plates E and F. Both of these Plates are thought to represent a transitional stage of vessels between typical arterioles and capillaries. They both contain a red blood cell rbc within their lumen l. The endothelial lining in both vessels and an endothelial nucleus en rest upon a basement membrane visualized at the end of the arrows. Primitive or poorly differentiated smooth muscle sm (also known as the perivascular cellular layer) can be seen sharing the basement membrane with the endothelium and in turn has been sheathed in its outer boundary by an early type of sarcolemmal sheath ss. Note the astrocytic processes ap and myelinated nerve fibers surrounding both vessels (x 7,281).

NOTE: Technical circumstances make it difficult to state with accuracy whether the tissues in Figure 28, Plates A, B, D and E come from normal or TETA-treated animals. Nevertheless, they are presented because they illustrate some classical features of neurons. Our inability to trace the origin of these tissues is further compounded by the presence of some mysterious, perfectly clear, extraneuronal spaces s. These spaces, because of their relationship to the surrounding neuropile and the fact that they are absolutely clear, could be considered artifacts. On the other hand, the well-preserved state of the other structures including those encapsulated within the spaces, coupled with the remarkable uniformity in configuration of these spaces, makes the artifact theory somewhat untenable. To the best of our knowledge there are no reports in literature of spaces quite like this, nor have we come across similar materials in other preparations.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D
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ss
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rbc
ap

FIGURE 29

ELECTRON MICROSCOPY OF RAT BRAIN - TETA-TREATED ANIMALS

NOTE: In all the animals from which the tissues for this Figure were obtained, cerebral edema was induced within 48 hours (acute cerebral edema) except for the tissue in Plate C where the original animal was treated for some 10 to 14 days.

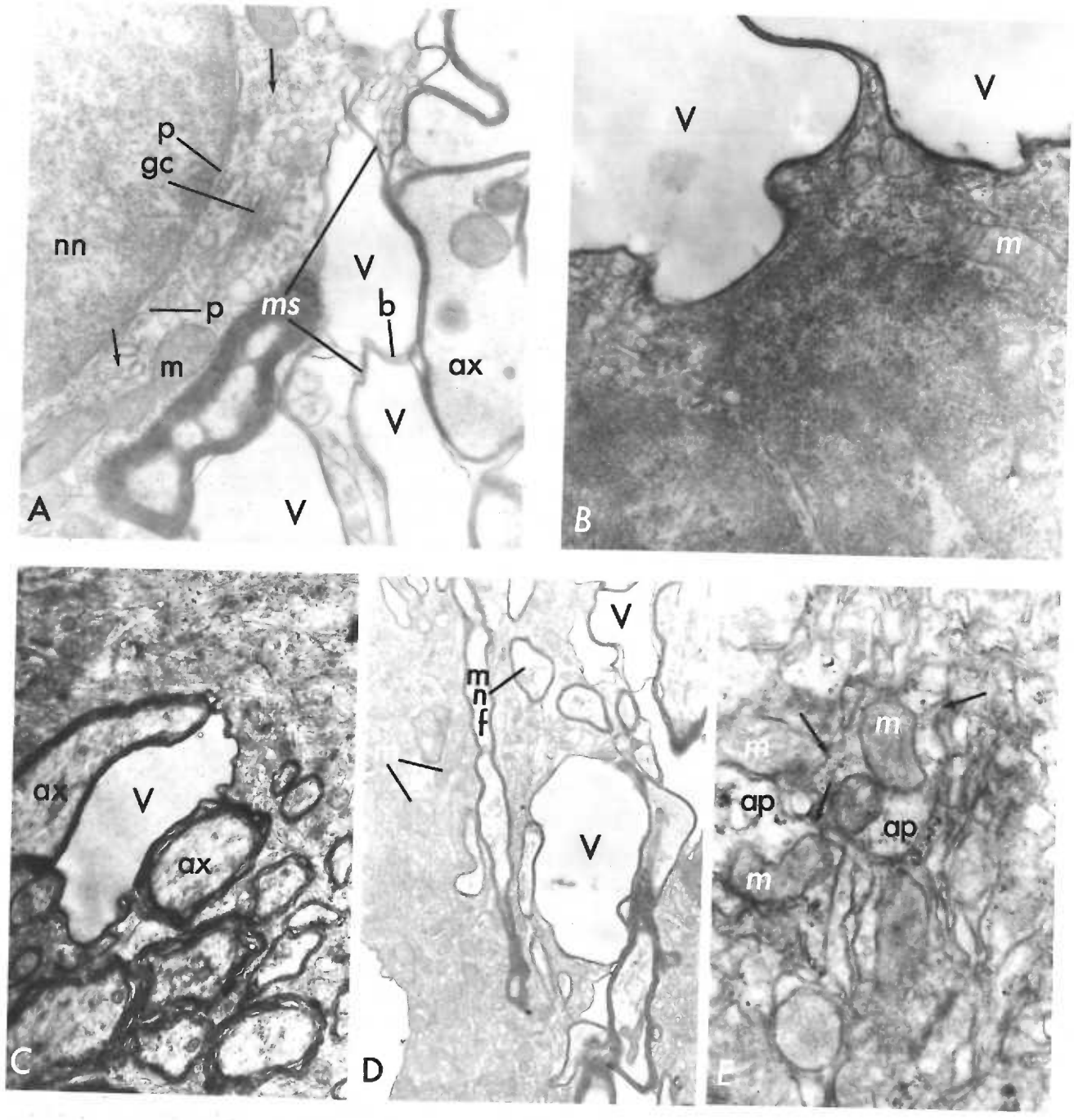
Plate A. Neuron with nucleus nn and cytoplasm, containing rosettes of RNA granules visible at the arrows and an array of membranes, canaliculi and vesicles probably part of a Golgi Complex gc. The double nuclear membrane is clearly evident as are the several pores p or fenestrations seen in its outer layer. The mitochondria m show seemingly a disorganization of their internal ridges or crests - not explainable by the plane of section - but yet inconclusive as far as evidence in favor of a pathological alteration. All the above structures lie next to large vacuoles V resulting from distention and/or splitting of myelin sheaths ms. The local blurring b seen at times in the lamellae of the distended myelin sheaths is probably due to their being stretched or twisted out of the plane parallel to the electron beam (2). An axis cylinder ax enveloped in a myelin sheath and containing mitochondria is also present in the field (x 20,462).

Plate B. Another example of adjacent distended myelin sheaths exhibiting vacuoles V. At least one mitochondrion m (this one elongated) with similar characteristics as the one described in Plate A can be seen. The rest of the neuropile cannot be accurately identified (x 20,462).

Plate C. Various myelinated nerve fibers lie next to a vacuole V, apparently formed by distention of the outer lamellae of the adjacent myelin sheath. This vacuole and all those described previously seem to contain particle-free fluid. They differ from the previously described unidentified extraneuronal spaces of Figure 28 by their enclosing membrane or coat formed by the outer myelin lamellae. Compact neuropile is seen in the upper part of the plate. Two of the axis cylinders ax are indicated (x 7,281).

Plate D. Again several vacuoles V are seen interspersed among compact normal-appearing neuropile (containing an abundance of mitochondria m) and several myelinated nerve fibers mnf. Note that there is no significant intercellular space (x 5,160).

Plate E. Again several irregular appearing mitochondria m larger than their counterparts of the same magnification observed in control brain tissue - are seen in astrocytic processes ap and in the surrounding neuropile. The astrocytic processes themselves seem to have a tendency toward becoming confluent with each other by destruction or disappearance of their limiting plasma membranes (see arrows) (x 14,502).


p
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m
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ap
ap
m
E

FIGURE 30

ELECTRON MICROSCOPY OF RAT BRAIN - CHRONICALLY TETA-TREATED ANIMALS

Plate A. An oligodendrocyte o with nucleolus ncl is seen between two astrocytes a (also containing nucleoli), one of which seems to indent it at the single arrow. One of the astrocytes (the lower one) has several gaps in its cytoplasm which communicate with the adjacent vacuole. The three glial cells are practically in a sea of vacuoles V. Some of the myelin sheaths are not only distended but seem to have broken and become confluent with each other. Myelinated axis cylinders ax are also present. The double arrows indicate area magnified in Plate B (x 5,160).

Plate B. Magnification of area in Plate A indicated by double arrows. The oligodendrocyte nucleus appears surrounded by a narrow rim of moderately dense cytoplasm oc which in contrast to the astrocytic cytoplasm ac appears grossly intact. The more watery astrocytic cytoplasm is broken off at least in one place and has become confluent with the adjacent vacuole V at the arrow (x 14,502).

Plate C. Magnification of area in Plate A indicated by single arrow. Again notice more dense, grossly intact oligodendroglial cytoplasm oc in contrast to more watery astrocytic cytoplasm ac which indents the former. As in the previous plate this astrocytic cytoplasm has at least one gap in its membrane (see arrow) by which it communicates with adjacent vacuole V (x 14,502).

Plate D. Another example of astroglial compression by adjacent distended myelin sheaths containing water-clear contents (x 5,160).

Plate E. A classical perineuronal oligodendrocyte next to normal appearing neuron. Neuronal cytoplasm nc shows characteristic elements. Both cells as well as surrounding neuropile appear intact. Neuronal nucleus nn is shown (x 7,281).

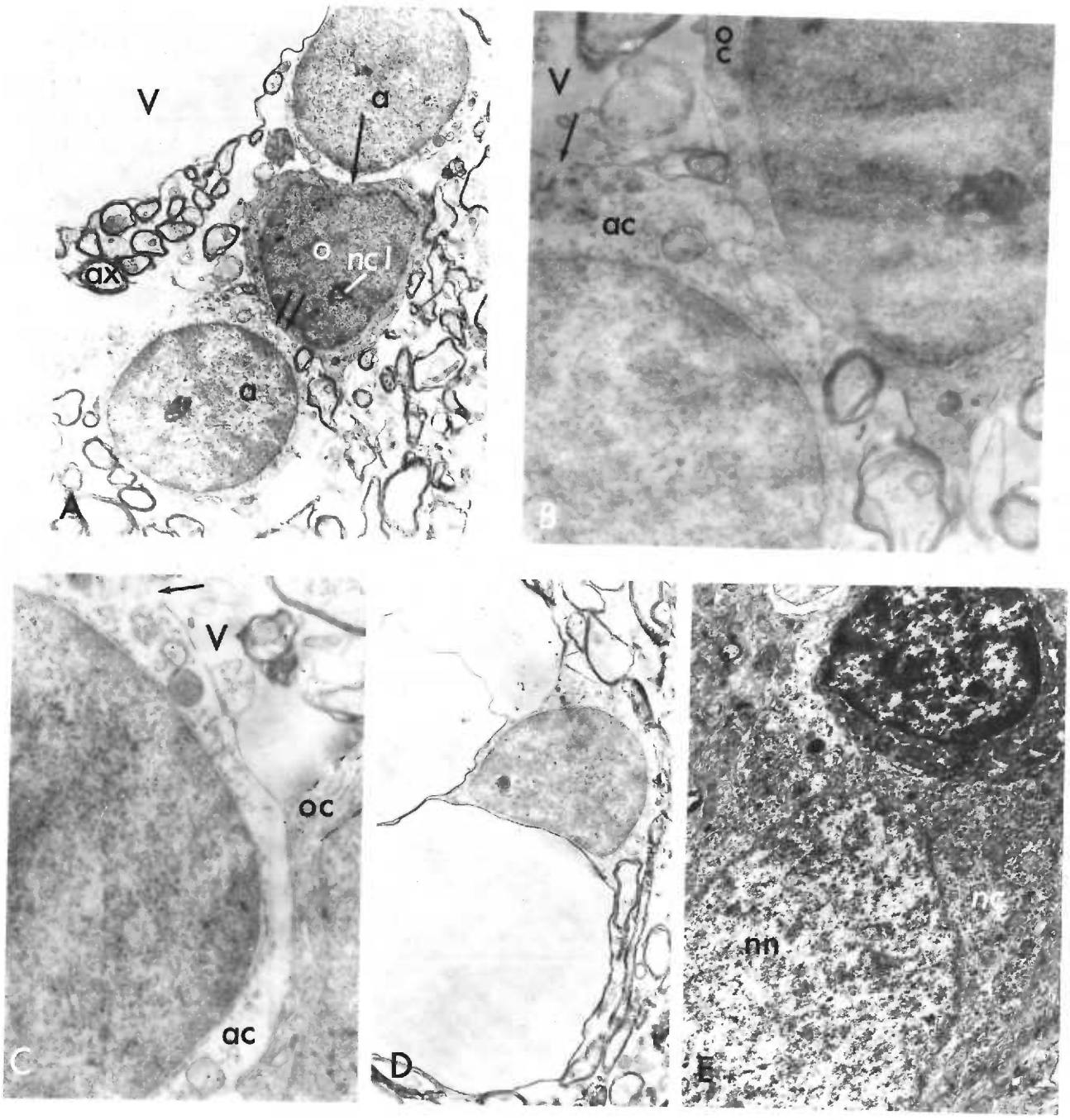

V
a
ax
ncl
a
A
V
oc
ac
B
V
oc
ac
C
D
nc
nn
E

FIGURE 31

ELECTRON MICROSCOPY OF RAT BRAIN TETA-TREATED ANIMALS

NOTE: Plates A, B, and C correspond to tissues from acutely treated animals, while Plates D and E come from chronically intoxicated rats.

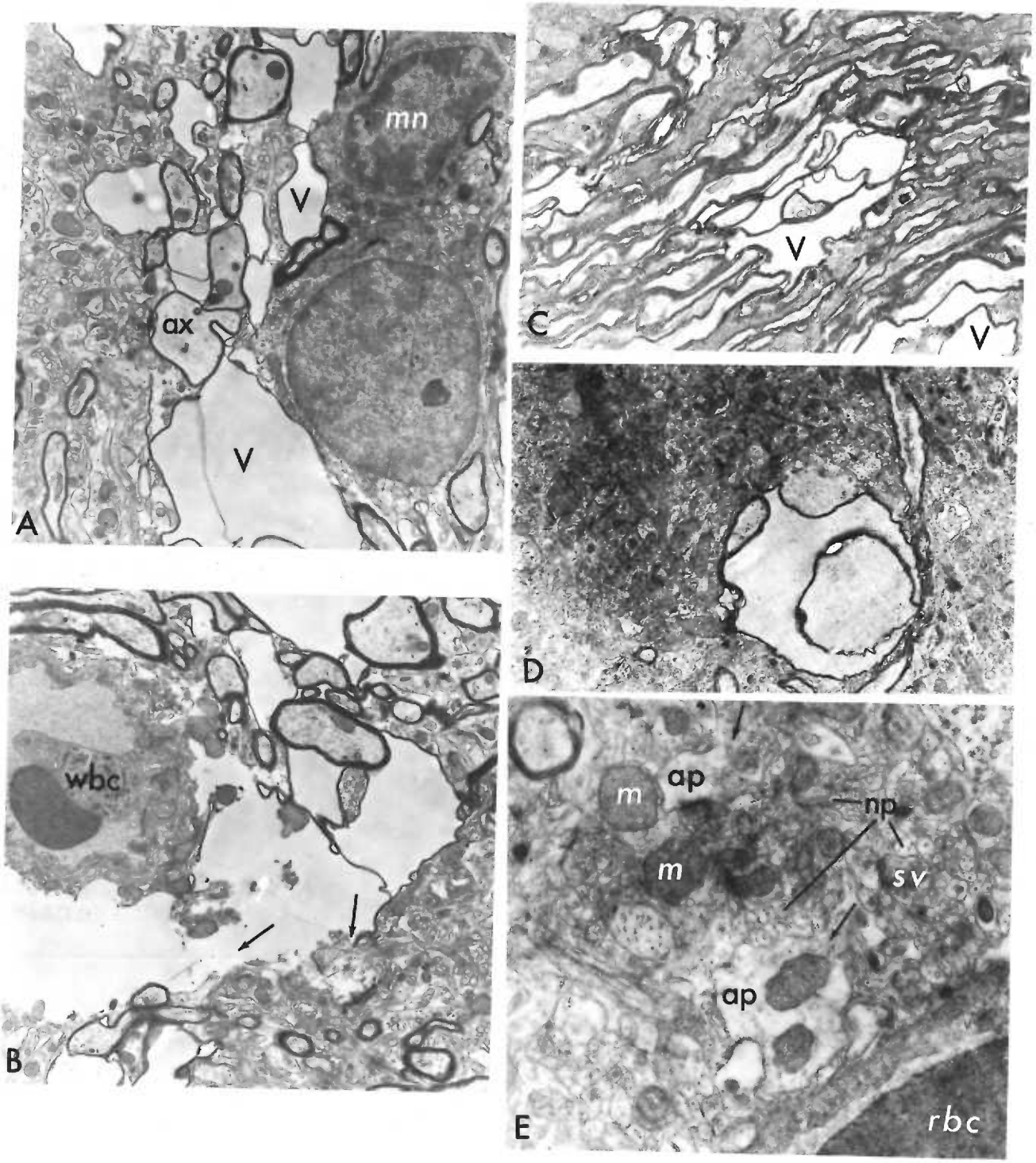
Plate A. Several vacuoles V are seen next to normal appearing neuropile and other myelinated nerve fibers (whose axis cylinders ax are indicated). Note neuron with nucleolus adjacent to atypical appearing cell, probably a microglia. Because of the relative decreased nuclear density of the microglial nucleus mn and cytoplasm, it is possible that this cell is undergoing pathological alterations (x 5,160).

Plate B. The distended myelin sheaths appear to have broken, forming confluent vacuoles (see arrows). Vessel immediately adjacent is somewhat deformed and contains red blood cell and probably a white blood cell wbc (x 5,160).

Plate C. The fairly compact structure of this animal's white matter is disrupted by the presence of several vacuoles V (x 5,160).

Plate D. Myelin sheath, in the midst of normal appearing neuropile, whose several lamellae seemed to have undergone distention in separate layers (x 5,160).

Plate E. Similar illustration as the one shown for Figure 29, Plate E, only that in this case the tissue is derived from an animal chronically treated with TETA. Several astrocytic processes ap with mitochondria are evident; in some there seems to be a disruption of their plasma membranes (see arrows). Some mitochondria m look enlarged and irregular. Small nerve processes np are everywhere. Red blood cell rbc inside vessel is seen in lower part of the Plate. Synaptic vesicle sv is indicated (x 14,502).


mn
V
ax
V
A
C
V
V
D
wbc
B
m
ap
np
m
sv
ap
rbc
E

DISCUSSION

The importance of cerebral edema in many pathologic processes affecting the brain has been emphasized. The number of monographs dealing with this subject documents it (76, 101). Furthermore, investigations of this phenomenon have not only uncovered many new areas of neuropathology, but have also provided insight into the many-faceted field of functional neurophysiology.

In the experimental laboratory, one of the major goals has been the production of cerebral edema under controlled conditions (34). Similarly, the absence of criteria universally agreed upon indicating the presence or extent of this "lesion" has hindered its understanding.

Discussion of the findings on triethyl tin cerebral edema is best done after division into various major categories.

A. Brain-water Content Determinations by Dehydration Techniques

A lack of standardization of procedures can account for many of the existing discrepancies among investigators in their evaluation of variations in brain-water content.

Our first study following the "classical" procedure for brain-water determination, using the dehydration method, yielded a value of 78.411 per cent for whole brain in rats, with an average body weight of 481 grams. This value is clearly within the range of values quoted in the literature for similar species (32, 149, 150). Yet, being within

the range is not enough when minor deviations from the control values signify critical differences as to whether or not cerebral edema is present. It was shown previously that after massive infusion of hypotonic solution with the development of clinical elevated intracranial pressure, the increase in brain-tissue fluid was only of the order of one to three per cent of the total fresh brain weight. Therefore, more important is the ability to find a similar amount of fluid in comparable areas from one single animal or from a series of animals matched for species, sex, age and body weight. In the study quoted above, the mean difference between opposite halves of the brain (A and B) when all four groups were pooled was 0.560 per cent. The range of mean whole brain-water content values for the same population was 0.724 per cent despite the fact that these Sprague-Dawley strain male rats only had a range of body weight of 24.5 grams, and were all of the same age.

Repetition of this study by the "modified" dehydration technique yielded a mean brain-water content, for similar rats weighing an average of 394 grams, of 77.783 per cent, also within the previously described range. This time, however, the mean difference between opposite brain halves (A and B) for the whole population was only 0.297 per cent. The range of mean whole-brain water content values for the same series of animals was 0.804 per cent, even though the variation in body weight was this time 152 grams.

The importance of sex, age, body weight and species in the variability of whole brain water content was recognized by Donaldson (31, 32,

33) and can be appreciated in Figures 32 and 33 which are reproductions from his original work of 1910 (32).

The ultimate purpose of research of this nature is the application of its principles and concepts to the human brain. Thus an actual study with tables such as that shown in Figure 33 which correlates the water content for man with the albino rat at corresponding ages, was a very important factor in the decision to select these animals for all subsequent phases of the study. For years no such observations had been reported in literature until in 1955 Graves and Himwich (53) published a study on the relationship between age and water content in various parts of rabbit brain. Even though this work was not as complete as Donaldson's, it set the stage for similar attempts (149).

Another point believed to be at the basis of many of the existing differences between investigators also involves the so-called "classical" methods of dehydration. The matter of excessive temperatures and prolonged intervals of dehydration for brain tissues is not new, as a matter of fact Shapiro (143) in 1939 thought that the values for the absolute brain water content seemed to depend on the method of dehydration. Similar claims were made by other authors (160, 186), yet they all seemed to remain unnoticed. The possible fractionation of lipids, especially those containing unsaturated fatty acids (when exposed to increasing temperatures) was described earlier.

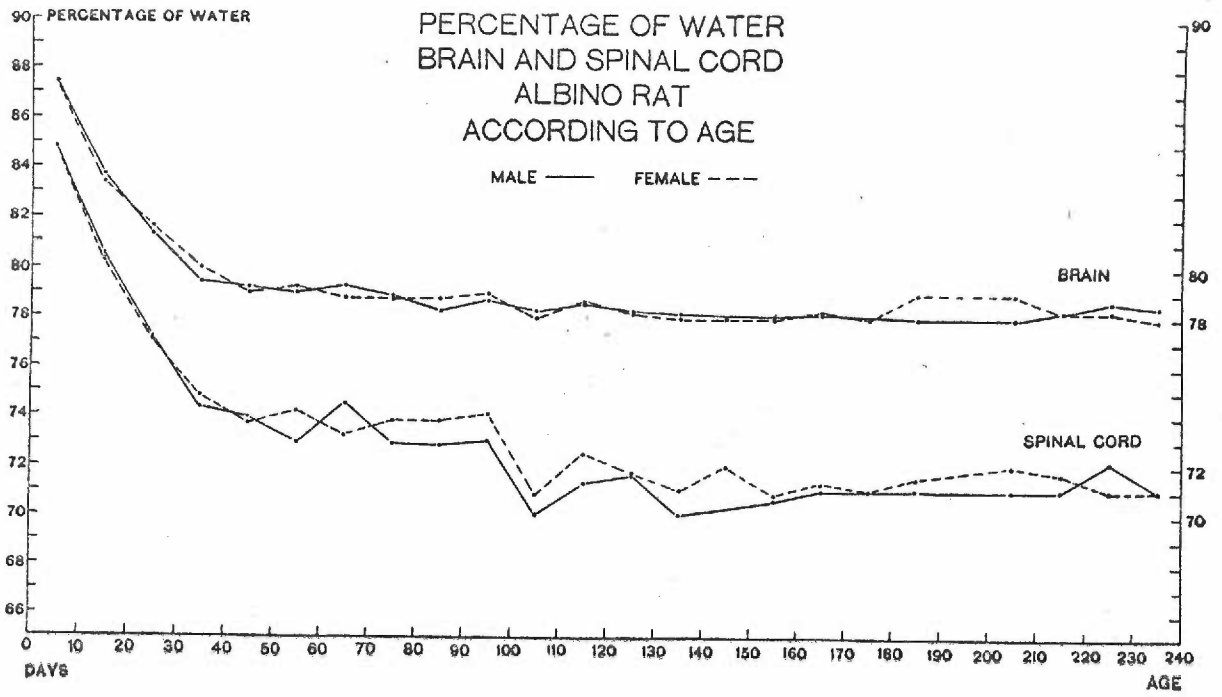
Inspection of Table 6 and Figure 3 in the Results section suggests that the criterion used by many investigators in determining the brain-water content which consists of heating the tissues until constant weight

FIGURE 32

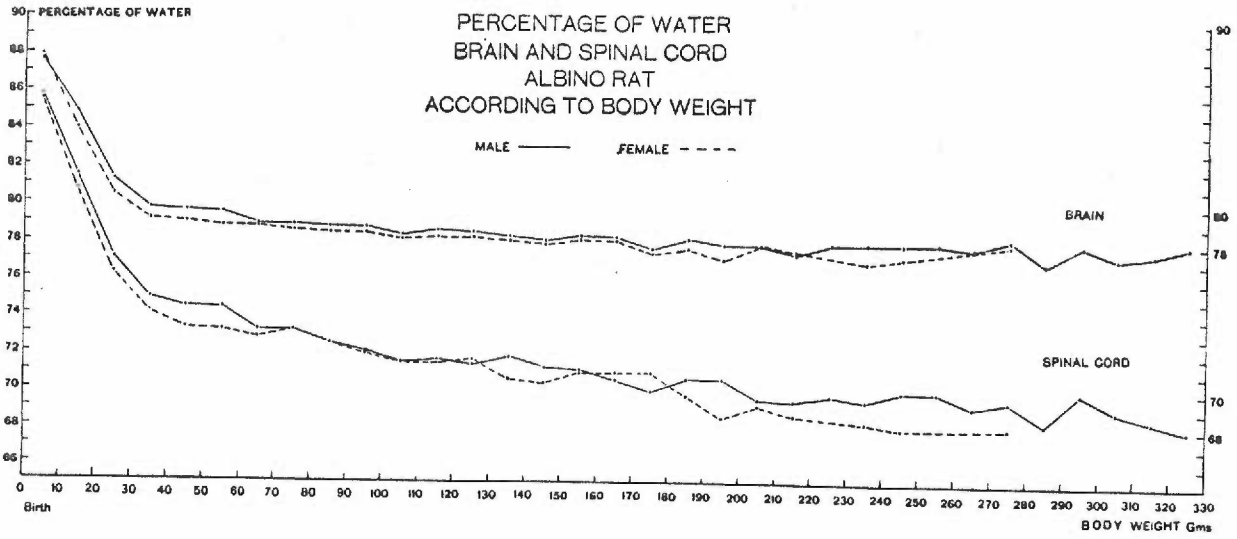
PERCENTAGE OF BRAIN AND SPINAL CORD WATER IN

ALBINO RAT ACCORDING TO AGE AND BODY WEIGHT

Both graphs are reproductions from Donaldson's original work of 1910 (32). They are based on information recorded in the upper two tables of Figure 33.



To show the changes in the percentage of water in the brain and in the spinal cord of the albino rat at different ages. The data for the two sexes are plotted separately. It is to be noted that the first entry is at the mean age of five days.



To show the changes in the percentage of water in the brain and in the spinal cord of the albino rat of different body weights. The data for the two sexes are plotted separately.

FIGURE 33

BRAIN WATER CONTENT IN THE ALBINO RAT

These three tables are also reproductions from Donaldson's original work of 1910 (32) The upper two tables give the actual figures for the graphs presented in Figure 32 (that is, the percentages of water in the rat brain according to age and body weight) and both are self-explanatory. The lower table compares the percentages of water in the brain of man and the albino rat at corresponding ages.

The mean values of the percentage of water in the brain and spinal cord of the albino rat. Both sexes arranged according to age, increasing by 10 day increments.[1]

AGE IN DAYS	PERCENTAGE OF WATER			
	BRAIN		SPINAL CORD	
	Male	Female	Male	Female
0–10	87.4	87.4	84.8	84.8
10–20	83.7	83.4	80.5	80.3
20–30	81.3	81.6	77.2	77.1
30–40	79.4	80.0	74.3	74.8
40–50	79.2	79.0	73.9	73.7
50–60	79.0	79.3	72.9	74.2
60–70	79.3	78.8	74.5	73.2
70–80	78.9	78.8	72.9	73.8
80–90	78.3	78.8	72.8	73.8
90–100	78.7	79.0	73.0	74.1
100–110	78.3	78.0	70.0	70.8
110–120	78.6	78.7	71.3	72.5
120–130	78.3	78.2	71.6	71.7
130–140	78.2	78.0	70.0	71.0
140–150		78.0		72.0
150–160	78.1	78.0	70.6	70.8
160–170	78.2	78.3	71.0	71.3
170–180		78.0		71.0
180–190	78.0	79.0	71.0	71.5
190–200				
200–210	78.0	79.0	71.0	72.0
210–220	78.3	78.3	71.0	71.7
220–230	78.7	78.3	72.2	71.0
230–240	78.5	78.0	71.0	71.0
240–250				
250–260				
260–270				
270–280				
280–290				
290–300	78.5		72.0	
300–310		77.4		68.2
310–320		77.3		68.0

[1] Note that the values here given begin with 0–10 days, i.e, a mean age of *five days after birth*. Hence the initial percentages are less than those in table 1 which gives the values at 5 grams, approximately the weight at birth.

The mean values of the percentage of water in the brain and spinal cord of the albino rat.[1] *Both sexes arranged according to body weights, increasing by 10 gram increments.*

BODY WEIGHT	PERCENTAGE OF WATER			
	BRAIN		SPINAL CORD	
	Male	Female	Male	Female
Grams.				
5	87.6	87.9	85.7	85.5
15	84.8	83.9	81.4	80.7
25	81.2	80.4	77.0	76.2
35	79.7	79.1	74.9	74.1
45	79.6	79.0	74.4	73.3
55	79.5	78.8	74.4	73.2
65	78.9	78.8	73.2	72.8
75	78.9	78.6	73.2	73.2
85	78.8	78.5	72.5	72.5
95	78.8	78.5	72.1	72.0
105	78.4	78.2	71.6	71.5
115	78.7	78.3	71.7	71.5
125	78.6	78.3	71.4	71.7
135	78.4	78.2	71.8	70.6
145	78.2	78.0	71.3	70.4
155	78.5	78.3	71.2	71.0
165	78.4	78.2	70.6	71.0
175	77.8	77.5	70.0	71.0
185	78.3	77.8	70.7	69.8
195	78.0	77.2	70.7	68.6
205	78.0	78.0	69.6	69.3
215	77.5	77.7	69.5	68.7
225	78.0		69.8	
235	78.0	77.0	69.5	68.3
245	78.0	77.3	70.0	68.0
255	78.0		70.0	
265	77.8		69.2	
275	78.3	78.0	69.5	68.0
285	77.0		68.3	
295	78.0		70.0	
305	77.3		69.0	
315	77.5		68.5	
325	78.0		68.0	

[1] For reasons similar to those previously given (see Donaldson '08, pp. 156–157), the individual records are not printed. These however are on file and copies of them may be had by application to the Director of the Wistar Institute.

Comparison of the percentage of water in the encephalon of man and the albino rat at corresponding ages

MAN		RAT	
Age Years	Percentage of Water	Percentage of Water	Age Days
Birth	88.3	87.7	Birth
2 years	81.1	81.3	26 days
9.5 years	79.2	78.6	115 days
25 years maturity	77.0	78.0	290 days

is reached, is inadequate. The mere fact that a tissue might weigh within .00025 grams in two separate recordings does not assure that it will stop losing weight on repeated heating. Exactly the reverse is found when careful successive weighings of the tissue are made. In both studies (Table 6 and 12, and Figures 3 and 6) the brain tissues continued to lose weight despite an interval of approximately 400 hours in one case and 150 hours in the other. In the first instance the temperature ranged from 110° to 116° C while in the modified technique it varied between 91° and 93° C.

The fixed interval of 18 hours was therefore used since it lies on a plateau portion of the time-weight loss curve (Figure 6. This time also agreed with the criteria set-up in trying to establish the "ideal" time interval of dehydration from the actual values (Tables 8 through 11).

Use of the "modified" procedure resulted in the existence of a narrow range of mean whole-brain water contents in the subsequent studies. In Figure 14 (acute TETA studies) the range for the control population was of the order of 0.3 per cent while in Figure 15 (chronic TETA studies) it was 0.5 per cent. The mean (A and B) difference (between opposite brain halves) for both control and experimental edema populations in the acute TETA studies was 0.2 per cent, while in the chronic animals it was 0.2 per cent and 0.3 per cent, respectively.

Finally, the advantages of using whole-brain preparations over single morphologically different brain areas are self-evident. For one thing, there is still much controversy over the areas of the brain which

preferentially develop edema. This difference of opinion is found even among investigators using the same type of experimental agent. For instance, Torack, Terry and Zimmerman (164) demonstrated with the help of illustrations, loosening of the fibers (with "vacuole" formation) of the corpus callosum and internal capsule in their triethyl tin-treated edematous mice-brain preparations. More recently, Aleu and others (2) repeated these experiments using rabbits and in this case they suggested that the subcortical white matter was involved, but the corpus callosum and internal capsule were spared. Similar contradictions are common throughout the literature.

Along the same line of thought, it is well known that there are differences in the integrity of the blood-brain barrier (and other types of barriers) in different parts of the central nervous system (13, 139). Thus, if one is dealing with any experimental material which is susceptible to the selective action of any of the brain barriers, examination of only a few single morphologically-different areas (since examination of all of these is not technically possible) might give a biased picture of the abnormal brain-water content relationships.

B. The Production of Cerebral Edema Using the Triethyl Tin Acetate (TETA) Experimental Model.

The consistency and dependability of this experimental agent ever since its introduction in 1955 has been repeatedly documented (2, 73, 95, 119, 157, 158, 144, 164). Furthermore, its selective action

on the central nervous system (apart from testicular atrophy after long exposure to alkyl tin) (95) is desirable in view of the advantages of controlling as many variable as possible in any experimental model. Similarly there is no evidence that neurons of the brain or spinal cord are damaged appreciably by it.

The changes observed in brain-water content secondary to acute or chronic TETA edema have been summarized in Figures 13, 14 and 15. In the acute triethyl tin series, the increase was of the order of 2.3 per cent while that of the chronic was 2.7 per cent of the total fresh brain weight. This difference was significant at the $p < .001$ level. It might also be noted that at no time was there any possibility of confusing the brain-water values of the experimental animals with the controls, for their respective ranges showed no overlap. For instance in the acute series the control figures ranged from 78.9 per cent to 79.2 per cent while in the experimental group they varied from 80.7 per cent to 87.4 per cent.

The variation in body weights in the acute TETA experiments was 44 grams for the controls and 51 grams for the treated animals. In the first series of experiments, (Results, section A, 1) in which the "classical" method of dehydration was used, the body weight range was 24.5 grams. Their range of mean brain-water content for all members of the population was 0.724. Were this "classical" procedure then to have been used in the acute TETA studies it is probable that the scatter of values in the treated and control populations would have made their difference less significant. A similar case can be made for the chronic TETA observations.

The increases in whole-brain fluid listed for the acutely and chronically treated animals may be placed in their proper perspective when it is remembered that there is only a 10 per cent difference in volumes between the cranial cavity and the brain substance. Therefore, with this limited "space" preventing further cerebral expansion, any small increase in the part of any one of the brain constituents will undoubtedly upset homeostasis and probably result in some degree of increased intracranial pressure. Incidentally, these increases compare very favorably with those reported by Magee and his group (95) in similar experiments.

When the edema induced in the acute and chronic experimental groups was calculated in terms of mean per cent swelling, this was found to be 11.1 per cent and 12.5 per cent, respectively, which also is comparable to the values reported by Streicher (158) and Katzman and his group (73).

No definitive comparison of TETA with other agents provoking cerebral edema is possible in this discussion in view of the extraordinary large number of them. A brief mention of some of their salient differences however might help emphasize the importance of alkyl tin edema in the field of experimental neuropathology. Not all of the methods of producing cerebral edema permit the evaluation of as many different parameters as are possible with triethyl tin. For example, "water intoxication" methods will produce significant increases in cerebrospinal fluid pressure, yet the extent to which systemic hypervolemia can be induced, without irreversibly damaging the experimental preparation, limits the degree of cerebral edema which can be elicited

(186). Among the traumatic methods, concussion is quite popular and yet throughout the literature gross discrepancies are commonplace as to whether significant water changes can be detected (112, 113, 186). Other traumatic methods such as cold or thermal injury are better suited for histological examination than for significant physico-chemical analysis (79, 85, 163).

Even among some of the "toxic substance" methods, like the one employing sodium cyanide (172, 182) the experimental set-up necessary for adequate results contrasts greatly with the simplicity observed when using the triethyl tin compounds. For instance, in the case of sodium cyanide artificial ventilation in the previously paralyzed animal is required.

The "ischemic" procedure involving bilateral carotid ligation is accompanied by a very high mortality as reported by Levine and Klein (86) who found that of 200 rats, 128 died within the first 24 hour period. Lastly, the disadvantages of the cerebral edema induced by procedures employing the implantation or allergic techniques, when a global approach to the pathology is contemplated, are all obvious.

Another consideration of the experimental set-up as used in this study involves its use in the evaluation of new therapeutic measures and drugs with possible application in the treatment of cerebral edema.*

*A few preliminary observations using Urevert®, dexamethasone (as Decadron®, and Papase® (a proteolytic enzyme) indicated that this experimental technique might be useful in detecting the ability of these drugs to control cerebral edema. Appropriate changes in both brain-water content and brain electrolytes suggested that possibility.

A study of that nature would also set the stage for the elucidation of the mechanisms of action of these various therapeutic agents.

C. Brain Electrolyte Changes in Cerebral Edema

Ever since Weed and McKibben (177) injected hypertonic salt solutions into experimental animals and showed an enduring fall in cerebrospinal fluid pressure, the question of osmotic pressure relationships across the brain capillary membranes in cerebral edema has been a subject of interest. Many of the theories proposed to explain the pathophysiology of cerebral edema assume the existence of a brain-barrier mechanism which actively pumps ions and other substances so as to preserve internal homeostasis in the water-ion compartment (29, 50).

The ions most usually studied are sodium, chloride and potassium. Technical difficulties prevented the evaluation of chloride in this project. Another cation, however, (calcium) was included after reports by Swank and Jackson (160) of changes in its brain concentration in hamsters following lipid meals. Some of the meals had induced convulsions and abnormal electrical discharges from the brain; this presumably could be due to hypoxia of brain tissues.

In both acute and chronic studies a gross increase in sodium and calcium was found. As far as potassium is concerned, the results were inconsistent because a minute but statistically significant rise was found in the acute series, while a similar rise (this time not significant) was found in the chronically treated animals. It is important to note that all results were expressed in milliequivalents per kilogram of

dry tissue as this permits a more clear appreciation of the absolute, undiluted electrolyte variation.

As evident from previous introductory material, both the sodium and potassium changes are consistent with the vast majority of reports dealing with all types of cerebral edema (2, 56, 73, 95, 156).

When the sodium values from this work are compared with similar values in TETA-edema preparations reported by other authors, these are found to be in agreement in both the acute and the chronic animal series. Magee and coworkers (95) reported a 49 per cent and a 36 per cent increase, respectively.

The 31 per cent and 49 per cent increase in calcium we found in the acutely and chronically-treated rats has no parallel in the literature since there are no published reports of brain calcium studies with alkyl tin-treated animals.

A curious finding observed only in the acute preparations may be seen in Figure 17. When the per cent increase in tissue sodium (from the control values) was plotted against the degree of cerebral swelling in the same tissues, a product-moment correlation (4) of 0.98 was found. This implies that the sole calculation of brain sodium concentrations could give an accurate estimate of the extent of tissue hydration or dehydration - or, as the case might be, an estimate of the degree of cerebral swelling present. The fact that this finding was not identified in the chronic TETA studies obviously poses doubts as to the veracity of the postulate.

D. Histological Findings in Cerebral Edema

The controversy which has arisen over this important aspect of cerebral edema was introduced earlier in this work. One of the major points of contention was the difficulty in correlating findings from electron and light microscopy. Many of the proponents for the continued acceptance of the classical concepts on neuroglia and the brain "intercellular space" base their evidence on conventional light microscopy observations. They furthermore refuse to accept the propositions suggested by the electron microscopists on the basis of what Sir Robert Hutchison (131) has termed evidence "the thinking mind instinctively rejects". The "newcomers" to this field, in turn, have not made enough allowances for their relative inability to systematically examine a whole brain area the way light microscopists, of course, can more easily attempt.

1. Light Microscopy Studies

The concepts on the nature of cerebral fluid accumulation in triethyl tin-treated animals have also undergone some change since their relatively recent inception. The original work of Magee and coworkers (95) on experimental triethyl tin intoxication reported a curious reticulation in the appearance of the white matter "consistent with an interstitial edema of the white matter". Smith and associates (144) on similar observations emphasized the myelin sheath separation by fluid-containing spaces and again asserted the presence of a "striking interstitial edema".

While our work was in progress an article by Aleu, Katzman and Terry (2) was published, containing findings almost identical to those demonstrated here. These consist of pronounced myelin sheath distention with vacuole formation, presumably due to the presence of edema fluid with compression of the surrounding structures. It is to be emphasized that the edema fluid identified was not interstitial but always lined by layers of myelin, the split most commonly occurring among the outer myelin lamellae.

A point of difference with the work of Aleu and associates (2) is that while they detected a sparing of the corpus callosum from the diffuse sponginess identified in the subcortical white matter, we found repeated involvement of white matter areas, including the corpus callosum. Our findings are supported by similar reports from Torack and coworkers (164). Two reproductions from their original work are presented in Figures 34A and 34B.

That a spongy appearance of the white matter is also characteristic of human cerebral edema is well demonstrated by Feigin and Popoff's (40) illustration in Figure 34D.

Other findings more in accord with the classical histologic interpretations of cerebral edema were those observed by the PTAH method. By these means, we have suggested some degree of astrocytic reaction in the form of swelling, irregularity and twisting of the astrocytic processes, and an increase in the neuroglial feltwork. Even though no cause and effect relationship can be postulated on these results, it is significant that an increase in brain fluids and electrolytes is associated with

this type of neuroglial reaction, in the presence of healthy-appearing neurones. Certainly there is some precedence in the work of Gerschenfeld and associates (50) who demonstrated swelling of the astrocytic cell bodies in in vitro studies of brain slices, to suggest such a contention.

Other reproductions from classical demonstrations of human cerebral edema are shown in Figures 34C and 35A, B, C, D, E and F. Note the surprising similarity between Greenfield's (54) demonstration of diffuse gliosis and clasmatodendrosis "in an edematous area remote from the tumor" (Figure 35D,) and our own observations in Figure 23, Plates E and F from the TETA-edema preparation.

2. Electron Microscopic Studies

The cerebral fluid accumulation as seen in the electron microscope and which was produced by the intraperitoneal administration or the ingestion of TETA appears to be mainly confined to the vacuoles resulting from myelin sheath distention. This finding agrees with the recent studies of Aleu and associates (2) in triethyl tin-treated rabbits. It contrasts though with the observations of Torack, Terry and Zimmerman (164) in triethyl tin treated mice, to the extent that their most important findings were an increase in the cytoplasmic volume for "clear glial cells" and a rupture of the plasma membranes of the glial processes. The latter investigators, however, make no mention of myelin sheath abnormalities despite the fact that their light microscopy illustrations look much like ours.

FIGURE 34

LIGHT MICROSCOPY OF CEREBRAL EDEMA

Note: Both plates A and B are reproductions from Torack, Terry & Zimmerman's The Fine Structure of Cerebral Fluid Accumulation. Am. J. Path., 1960. 36, 273-288.

Plates C and D are from Feigin and Popoff's Neuropathological Observations on Cerebral Edema. Arch. Neurol., 1962. 6, 77/151-86/160.

A. Intact mouse cerebrum showing normal compact structure of the corpus callosum (CC), fimbria (F) and internal capsule (IC). Hematoxylin and eosin stain.

B. Mouse cerebrum in chronic triethyl tin poisoning. There is loosening of the fibers of the corpus callosum (CC), fimbria (F) and internal capsule (IC). Hematoxylin and eosin stain.

C. Section from arcuate white matter. The astrocytes are not entirely normal. Cajal stain.

D. Edematous white matter adjacent to carcinomatous module. The tissues show marked spongy change. Naoumenko's modification of Cajal stain.

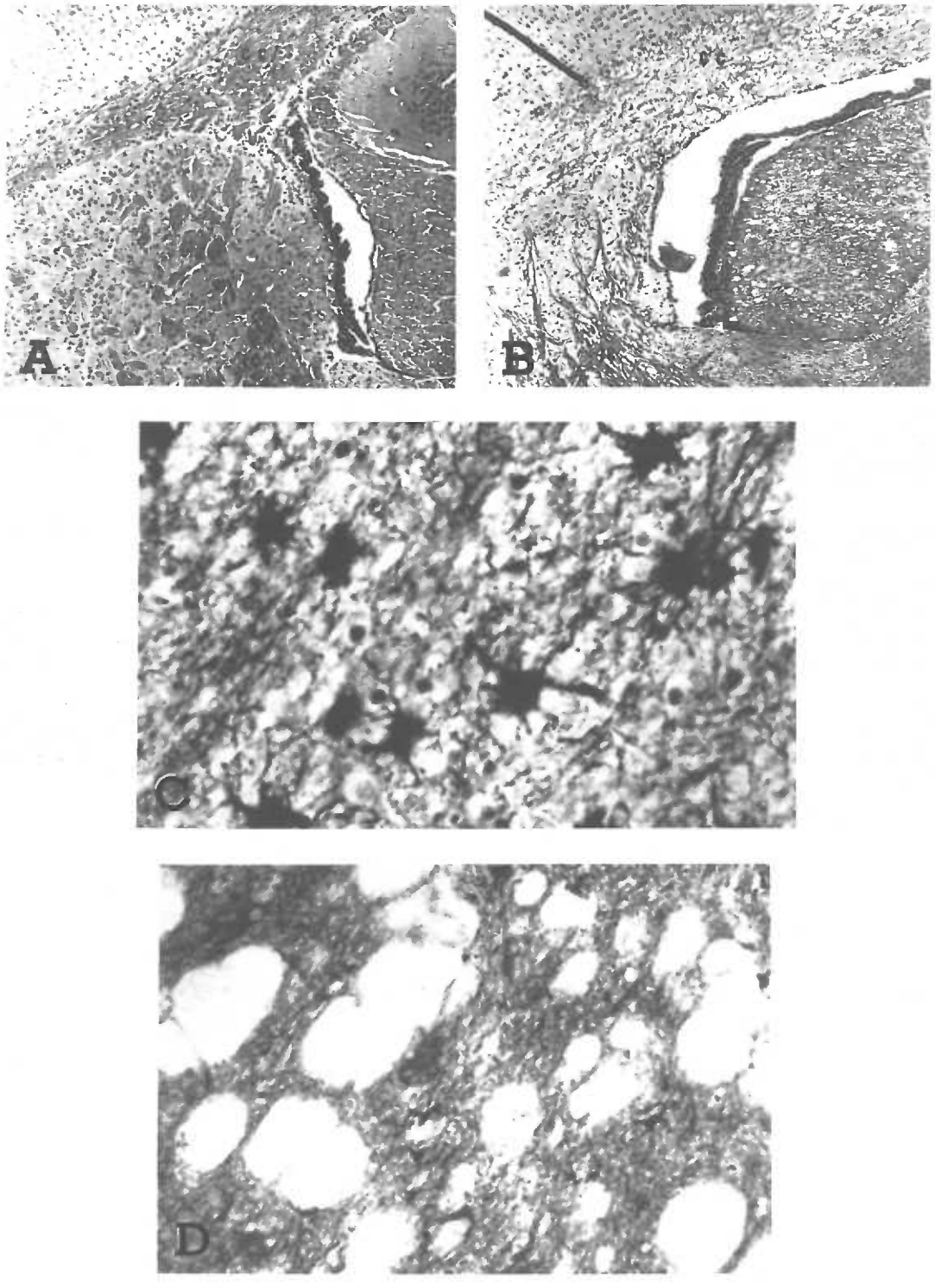

A
B
C
D

FIGURE 35

LIGHT MICROSCOPY OF CEREBRAL EDEMA

Note: Plates A, B, C and D are reproductions from Greenfield's Cerebral Edema Associated with Intracranial Tumors. Brain, 1939. 62, 129-152.

Plates E and F are from Feigin & Popoff's Neuropathological Changes Late in Cerebral Edema. J. Neuropath. Exp. Neurol., 1963. 22, 500-511.

A. Secondary nodules in right frontal lobe from a renal carcinoma. The myelin sheaths are irregularly swollen and many granules of lipoid lie free in the tissues (Weigert-Pal counterstained alum carmine).

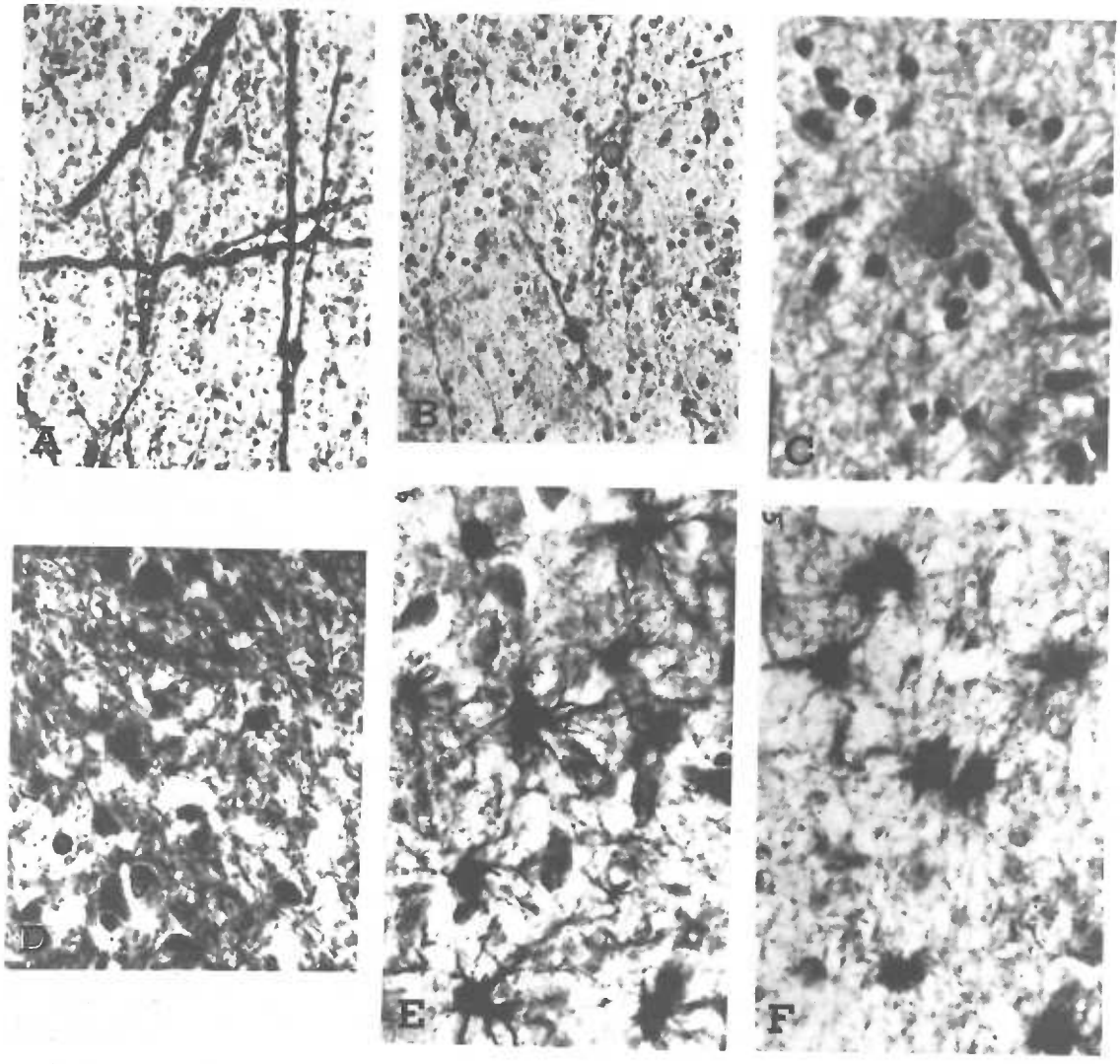
B. Right occipital glioblastoma. The myelin sheaths in the most edematous area are very scanty and those present show many bulbous swellings on their course or at their terminations (Weigert-Pal counterstained alum carmine).

C. Fronto-vertical endothelioma. A swollen binucleated astrocyte is seen with much overgrowth of neuroglial fibers (Mallory's phosphotungstic acid hematoxylin stain).

D. Astrocytoma of left parietal lobe. Diffuse gliosis (and clasmatodendrosis) in the edematous area remote from the tumor (Mallory's phosphotungstic acid hematoxylin stain).

E. Normal arcuate white matter. Astrocytes are normal in appearance (Paraffin-gold sublimate stain).

F. Deep white matter, hypertensive case. The astrocytes are abnormal with short poorly stained processes (Paraffin-gold sublimate stain).


A
B
C
D
E
F

The finding in this study of possible abnormalities in the mitochondria of astrocytic processes coupled with breaks in their plasma membranes has precedent in the work of Torack and associates (164) and some other authors (37, 102). Yet, a reconciliation between their observations and ours is not simple unless the matter of degree of cerebral edema is introduced. This of course is hindered to a certain extent since they do not present any corresponding data of brain-water or electrolyte content or alternate physical or chemical measurements.

The vacuolar spaces studied contained a particle-free, water clear material (presumably edema fluid) which is consistent with other published reports (2, 164) and with Smith and coworkers' (144) classification of triethyl tin edema, among those of the transudative type.

Our work further documents the "modern" belief of a minute and insignificant extracellular space, even in the case of pronounced brain fluid accumulation. Nor were we able to identify perivascular spaces (of the Virchow-Robin type) in either normal or experimental material. This is similar to the findings of Maynard and associates (98) in their extensive studies of the vascular bed of the rat cerebral cortex.

As pointed out in the pertinent section of Methods and Materials, consistency in the identification of normal structures visualized by electron microscopy demands that an investigator align himself with one of the schools of thought existing on this issue. This emphasizes the lack of correlative studies between light and electron microscopy observations and the need to "bridge the gap".

Another problem inherent in studies of this kind is the matter of how representative a tissue sample really is of the area it is supposed to demonstrate. Very exacting techniques hinder accurate localization or tagging of the tissue blocks so that different investigabors might be reporting their observations from different structural areas. This again would impede satisfactory comparison of their work. the infinitesimal nature of tissues dealthwith makes this proposition not too unlikely.

E. Mechanisms of Action of the Triethyl Tin Compounds

The typical symptomatology and associated body changes seen in our TETA-treated animals have been previously described in the literature (20, 95, 102, 103). The reason for their appearance is not yet well understood and only a few of the basic biochemical derangements have been identified.

The earliest discovery was the realization that the triethyl tin salts uncoupled oxidative phospherylation *in vitro* and also inhibited brain respiration both *in vivo* and *in vitro*. Like other uncoupling drugs such as thyroxine they were found to produce mitochondrial swelling at lower concentrations than those at which they were able to uncouple oxidative phosphorylation. Unlike 2,4 dinitrophenol, however, they inhibited mitochondrial adenosine triphosphatases. A general reduction in metabolism was also consistent with the fall in body temperature observed in the triethyl tin-treated animals (103).

Moore and Brody (102) later suggested that because a portion of the triethyl tin molecule in solution exists as a cation, it was possible

that it could accept electrons along the electron transport chain and thus divert their passage or set up an alternate route for electron flow. This new pathway could exclude a step in the chain normally associated with energy-rich phosphate transfer.

Cremer (20) then proceeded to show that triethyl tin sulfate was an active inhibitor of creatine phosphate synthesis in brain slices; furthermore, the organic compound appeared to be more sensitive to triethyl tin than to oxygen consumption.

A finding of utmost importance in explaining the basic biochemical aberrations in alkyl tin edema resulted from studies with Na^{24}. It appears that the earliest change produced by triethyl tin *in vivo* is a stagnation of the sodium pools of the cerebrospinal fluid and brain. This could result from an inhibition of the supply of energy necessary to maintain the mechanism which controls cerebral salt and water balance (20).

It is interesting that the alkyl tin compounds do not inhibit anaerobic glycolysis of brain homogenates, in view of the work of Streicher, Prokop and Klatzo (159) who were unable to produce triethyl tin edema in immature rats and cats. It is well known that in the presence of anoxia the very young and immature animals are capable of utilising quickly and efficiently the anaerobic breakdown of glycogen to satisfy the energy requirements of their central nervous system (149).

Also it has been suggested that the prolonged period of central nervous system depression shown by the triethyl tin-treated animals

could be related to a centrally-mediated release of catecholamines from the brain and adrenal medulla (103).

SUMMARY AND CONCLUSIONS

An experimental model of the clinical condition was utilized to study cerebral edema. This was accomplished by the use of triethyl tin acetate, which was administered either intraperitoneally or by inclusion in the rat's food and water depending on whether acute or chronic cerebral edema was to be induced. This model is thought to be extremely practical not only because of its consistency and dependability - as documented in this work - but also because it allows the simultaneous observation of several key parameters.

A preliminary study of the classical dehydration technique for the determination of brain-water content resulted in a more reliable and, possibly, a more valid modification of that procedure.

Cerebral edema, then, as produced in this investigation, was characterized by: a) an increase in brain-water content; b) a calculated "cerebral swelling" of 11% to 12.5% in acute and chronically intoxicated animals, respectively; c) typical symptomatology and associated body changes; d) changes in brain-tissue electrolytes, which consisted of a 33% to 36% increase in sodium, a 31% to 49% increase in calcium, and a 3% to 2.5% increase in potassium for acute and chronically treated animals; and e) by distinctive histological findings. These relied on both light and microscopic observations indicating myelin distention with vacuole formation which seem most certainly due to the presence of edema fluid. These two microscopic methods also suggested that a

neuroglial reaction occurs in the course of cerebral edema with the astrocytes being the probable incriminating cell structure.

Evidence presented for the absence of a significant intercellular space in the normal brain coupled with a similar finding in the model cerebral edema is consistent with current opinions on the subject. The histological observations have been accompanied by an attempt to demonstrate base-line normal structures by electron microscopy.

This work would indicate a more important function for the studied neuroglia than their mere role as supporting tissue of the central and peripheral nervous system. Certainly the lack of a significant cerebral extracellular space emphasizes the need to postulate some other anatomical location for the water-ion compartment. Furthermore, the nature of the physico-chemical changes detected in the experimental model suggests that in cerebral edema there must occur an impairment of the mechanism which controls cerebral salt and water balance.

Finally, it is suggested that this experimental model might be useful in the possible evaluation of new therapeutic measures and agents to be used in the treatment of cerebral edema.

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APPENDIX

COMMONLY USED ABBREVIATIONS

BBB - blood-brain barrier

BLB - blood-liquor barrier

BW - body weight

CA - carbonic anhydrase

CNS - central nervous system

CSF - cerebrospinal fluid

DIBV - differential index of brain volume

FAB - formol ammonium bromide (bromformalin)

ICP - intracranial pressure

ip - intraperitoneally

LBB - liquor-brain barrier

oligo - oligodendrocyte

PAS - purified protein derivative of tuberculin

ppm - parts per million

PTAH - phosphotungstic acid hematoxylin

TETA - triethyl tin acetate

CALCULATION OF ELECTROLYTES FROM TISSUE SAMPLES (using flame photometry)

Formula = $\frac{\text{Unknown}}{\text{Standard}}$ x concentration

where unknown is the reading of the sample to be measured.

Standard is the reading of stock solution (after adequate dilution).

Concentration is the original concentration of stock solution.

Example: Measurement of Sodium in a brain-tissue sample

Tissue weight prior to ashing = 0.11647 grams
Unknown reading = 51
Standard reading = 50
Original concentration of stock solution = 150 mEq/L
Dilution of stock solution to make standard = 1/100
Dilution of ashed tissue sample = diluted to 25 cc

Therefore $\frac{51}{50}$ x 150 = 153 mEq/L or 153 x 10^{-3} mEq/cc

(and because of stock dilution) = 153 x 10^{-5} mEq/cc

(since sample was diluted up to 25 cc, multiply by 25) = 3,825 x 10^{-5} mEq

Then using simple ratio:

If 3,825 x 10^{-5} mEq of Na^+ are present in 0.11647 grams of tissue

X mEq of Na^+ are present in 10^3 grams of tissue

$$\frac{3.825 \times 10^{-5}}{0.11647} = \frac{X}{10^3}$$

X = 328.411 mEq/kg of dry tissue, which is the sodium concentration in our tissue sample