Drugs for the Treatment of Traumatic Brain Injury

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I. INTRODUCTION

The leading cause of death for individuals under 45 years of age in the United States is the result of accidents. Accidents are the fourth leading cause of death when all age groups are considered following heart disease, cancer, and stroke.[1,2] The single most common determinate of accidental mortality is vehicular-related.[1,2] Each year, over 52,000 Americans die in automobile accidents. Rather similar results are reported in most developed countries.[3] While the relationship of mortality to brain injury is well recognized, it is less well known that the morbidity following traumatic brain injury is the primary cause of persisting disability of those who survive. From a 1974 survey, it was estimated that survivors of traumatic brain injury required nearly 7 million

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days of hospitalization and accounted for the loss of almost 10 million days of normal activity.[1,2] The lifetime cost of medical care for a patient with serious brain injury can be very great,[1] exceeding the limit of most medical insurance policies.

There are other types of insults to the brain that exhibit pathological and often life-threatening increases in the tissue-water content of the brain that are similar to those resulting from traumatic head injury.[1,2] Among these insults are ischemic stroke, cardiac arrest, arrested breathing, cerebral embolism, Reye's syndrome, encephomyelitis, hydrocephalus, post-operative brain trauma, the neurological aspects of AIDS, spinal injury, and even brain concussions.[4–7] Of these insults, ischemic stroke is the third leading cause of mortality in the United States, accounting for at least 200,000 deaths annually and the hospitalization of 400,000 patients.[8,9] There are about 1.8 million patients who have survived one or more strokes and these are those most likely to experience subsequent strokes.[8,9] In view of these statistics, it is not only lamentable that there is no specific treatment for these medical problems but rather incredible that no concerted effort has been made to discover and develop drugs for this purpose.

II. THE CONCEPTION OF BRAIN INJURY DRUGS

At the onset of our research in this area, there was only a fragmentary understanding of the molecular mechanism of brain injury, correlating the cause and resolution of the attendant cerebral edema associated with altered cerebrocapillary permeability. Since specific therapeutic agents were not available, only non-specific drugs had been examined for this purpose and some of these are in current use. The apparent purpose for some of the most commonly used agents was to control brain volume as generally reflected by increased intracranial pressure (ICP). These drugs include the obvious osmotic,[10–16] loop,[10,12,17–20] and carbonic anhydrase inhibitory[21–23] diuretics. For obvious reasons related to brain trauma, the use of steriods[24–26] and barbiturates[27–31] is generally a part of the non-specific therapeutic regimen. The clinical usefulness of diuretics is limited by the electrolyte loss, blood volume decrease, and plasma pH changes. The coma induced by barbiturates poses a drawback to this therapy and the clinical usefulness of steroids is questionable.[25]

Dr. Edward J. Cragoe, Jr. *received his B.A. in chemistry at Baker University. His M.A. and Ph.D. degrees in organic chemistry were received at Nebraska University where he also served as a National Defense Research Commission fellow. He began his career as a research associate at Sharp and Dohme which later became the Merck Sharp and Dohme Research Laboratories. For a while, he also served as a chemistry instructor at Pennsylvania State University. He was promoted through various levels of research management to Senior Director of Medicinal Chemistry and finally was one of only two to be appointed to the position of Distinguished Senior Scientist at Merck. He has authored about 230 scientific papers, 24 books or chapters in books, and holds 260 patents. He has made contributions in over 20 areas of medicinal chemistry including outstanding contributions in the fields of diuretics, brain injury drugs, and ion transport inhibitors.*

A. Rationale for New Therapeutic Agents

Evidence has been presented for a correlation of pathologically altered fluid and solute compartmentalization in the injured brain with the subsequent morbidity and mortality.[32] Unfortunately, most of the studies regarding pathological fluid compartmentalization in the injured brain have focused on cerebral white matter, which accounts for 50% of the brain volume, as the site of clinically relevant cerebral edema.[33a] However, studies focused on pathologically altered fluid compartmentalization within the gray matter, which also constitutes 50% of the brain volume, reveals this site to be of major clinical importance. In fact, since neuronal damage is associated with this site, it may constitute the most significant area affected. Vasogenic edema results in a net gain in water, which collects mostly in white matter where the path of intracellular space is rather tortuous. The focus of our study was on cellular or cytotoxic edema, which appears to be specifically associated with traumatic brain injury and its main symptom is astroglial swelling. The brain need not show appreciable net gain in water content in cellular edema; however, cellular and vasogenic edema can coexist.[33b]

Subsequent to blunt injury and stroke, edema restricted to astrocytes located within the complex cellular mosaic of gray matter is observed.[34–38] Virtually the entire surface area of nutrient cerebrocortical capillaries is covered with the cellular extensions of astrocytes which are physical satellites of the neuronal perikarya. Since astrocytes normally constitute 20–25% of the volume of the cerebral cortex,[39] they are strategically located to alter the concentrations of critical capillary-borne solutes, gases, and nutrients to and from neurons. Even modest cerebrocortical tissue edema predominately limited to swollen astrocytes significantly alters capillary tissue microgeometry to the possible detriment of capillary tissue solute transfer.[40] Astroglial swelling and the concomitant increase in Na^+ content affects Na^+-dependent uptake and results in the inactivation of transmitters.

At the time that the search for new brain injury drugs was initiated, the *in vivo* studies conducted at Albany Medical College demonstrated that K^+ and/or a putative neurotransmitter-dependent, HCO_3^--stimulated, net coupled Cl^- plus cation transport associated with an osmotic equivalent of water was responsible for the tissue slice swelling which, in turn, was predominately associated with astroglial swelling. Among the putative neurotransmitters are adenosine and norepinephrine.[41] Furthermore, several loop diuretics, such as ethacrynic acid and MK-473 known to block chloride transport in the thick ascending limb of Henle's loop, were found to inhibit swelling and chloride transport in the *in vitro* cerebrocortical slice preparation,[42–44] cultured rat astrocytes,[42] and in the intact superfused cerebral cortex *in vivo*.[45] Finally, early studies[20,46] revealed that the administration of these compounds after experimental and clinical head injury resulted in decreased mortality and morbidity as compared to controls.

B. Chloride Transport Inhibitors as Specific Agents for Brain Injury Therapy

The logical objective of our studies was to design compounds with specific activity in inhibiting chloride transport related to cerebral trauma and the

concomitant edema which were also devoid of salidiuretic effects. Our extensive experience with compounds of the (indanyloxy)alkanoic acid series[47] which were known to block renal chloride transport[48–50] suggested that this class of compounds represented an area of high potential for exploration. The availability of an *in vitro* assay which measured intrinsic anti-brain edema activity permitted both the screening of various ion transport inhibitors (i.e., loop diuretics) and new compounds designed and prepared as potential agents for the treatment of brain injury.

III. BIOLOGICAL ASSAYS

A. In Vitro Assays

1. The In Vitro Cat Cerebrocortical Slice Assay

This test has been described in detail[44,51–54] and has proved to be of great value in determining the intrinsic activity of test compounds. By developing a concentration/response curve for inhibition of the swelling of the slices, one could observe the inhibitory characteristics of each compound. It was interesting to note that the sensitivity to the transport inhibitors varies markedly with the species from which the slice is taken. The tissue source for the general screening assay is the cat, which is highly sensitive to chloride transport inhibitors and was the species used in the acceleration-deceleration studies. As will be shown later, the cerebrocortical slices from the guinea pig are also quite sensitive to these inhibitors, but slices from the laboratory rat are quite insensitive.

The concentration/response curves reveal other important features of the test compounds. Many curves are U-shaped, demonstrating that the higher concentrations are less inhibitory than the lower concentrations. There is evidence that the effects observed at the high concentrations are due to inhibition of the (Na^+,K^+)-pump. Some compounds never achieve 100% inhibition and others display sharp V-shaped curves. Obviously, the most attractive compounds for use as drugs are those that display broad L-Shaped or U-shaped curves with complete or nearly complete inhibition over a wide concentration range.

2. The In Vitro Primary Rat Astrocyte Culture Assay

This test was developed[39,42] and employed to demonstrate that the ion transport process and the resulting swelling were primarily located in astrocytes. However, the insensitivity of rat tissues to inhibitors of the type described relegates this assay to a position of little value as a screen. Undoubtedly, primary astrocyte cultures from other species could be developed.

B. In Vivo Assays

1. The Acceleration-Deceleration Assay in Cats

This model has been described in detail.[46,51,55] Although a variety of other animal models of brain injury have been described, none of them exhibit appreciable correlation with human concussive brain injury. Experimental

biochemical studies have shown that concussive brain injury results from subjecting the skull-encased brain to alternating acceleration/deceleration forces. It has been demonstrated[56] that the imposition of a period of controlled post-traumatic hypoxia following acceleration/deceleration brain injury in the cat results in prolonged coma with delayed death similar to that observed in clinical experience. Therefore, this experimental paradigm of head injury constitutes an *in vivo* model for assaying administered drugs for their effectiveness in altering mortality and neurological morbidity.[46] Accordingly, anesthetized, conditioned animals are exposed for 63 s to rapid (1,350 oscillations/minute) acceleration/deceleration translational motion with an average peak magnitude of 78 G. After 40 min, this is followed by a 60 min period of hypoxia using 6% O_2 at a pO_2 of 23 torr.[46] Since the mortality of untreated control animals varies with sex and season as shown in Figure 1, paired control and treated animals are always run in parallel each day.

This assay is generally employed for determining the effects of experimental drugs on mortality; however, neurological recovery can also be readily evaluated.[51] Several compounds were evaluated in the *in vitro* cat cerebrocortical slice assay in the early stages of the study. Criterion for the selection of compounds for the *in vivo* assay were its reported use in the clinic or the observation of reasonably good activity in the cerebrocortical slice assay. From the data recorded in Table I, it can be seen that acetazolamide and methylprednisolone sodium succinate were inactive at the doses tested, even though methylprednisolone is frequently employed clinically. Although both ethacrynic and indacrinone (MK-196) consistently reduced head injury related mortality, statistical significance could not be determined with the number of animals used in the study. These findings appear to reflect, to some degree, the intrinsic biological activity of these compounds as determined by the *in vitro* cerebrocortical slice assay. Of equal importance is the fact that the data also reflect the bioavailability of the drug at relevant sites in the brain. Indeed,

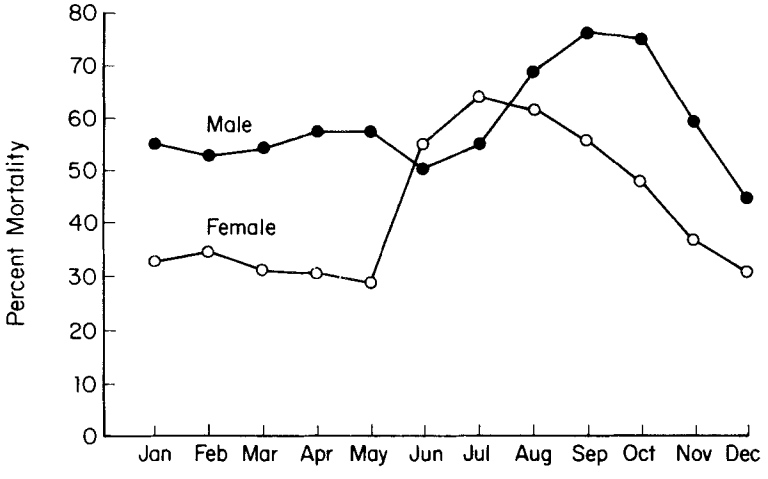


Figure 1. The mortality rates of 257 head-injured, control cats grouped by month of year and sex. The data are expressed as a weighted moving average with an average of 12 animals per data point.

Table I.
In Vivo Activity of Various Agents in the Acceleration-Deceleration Head Injury Assay

Agent	I.V. Dose mg/kg	Treated Animals Deaths/ Total	Treated Animals % Mortality	Control Animals Deaths/ Total	Control Animals % Mortality	Δ %
Acetazolamide	1	8/16	50	8/16	50	0[a]
Methylprednisolone Sodium Succinate	30	9/18	50	10/20	50	0[a]
Ethacrynic Acid	1	14/39	36	19/37	51	15[a]
Indacrinone	1	8/22	36	11/22	50	16[a]
MK-473	1	11/42	26	19/37	51	25[b]

[a]Not significant.
[b]$p = <0.05$ for each study.
Pairs were matched according to sex and run simultaneously with one animal randomly chosen to receive the intravenously administered drug and the other used as a control. Deaths were recorded over a period of 24 h following trauma, excluding the animals that died prior to application of hypoxia. Δ % refers to differences in percent mortality between control and treated animals.

the *in vivo* evaluation of these drugs involves penetration of the blood-brain barrier, a constraint not required for agents which do not act on the brain or for those studied in the *in vitro* assay. It is interesting to note that there is clear evidence that ethacrynic acid significantly ($p < 0.025$) improves both the rate and ultimate level of neurological recovery in cats surviving the head-injury insult. These data, as seen in Figure 2, may help to explain the clinical results obtained with this drug as described later.

2. *In Vivo Inhibition of Astrocytic Swelling*

This assay is not generally used in evaluating drugs, but rather to demonstrate that the swelling of cerebrocortical tissue slices observed *in vitro* are also seen *in vivo* and that drugs that inhibit swelling *in vitro* can also be effective *in vivo*.[45] The exposed and intact cerebral cortices of cats *in vivo* were superfused under normothermic conditions with isotonic artificial CSF containing added 0.125 mM adenosine, which is a stimulus for cerebrocortical tissue slice swelling *in vitro*.[42] This was found to result in significant ($p < 0.01$) chloride-rich cerebrocortical swelling (75 μL/g) that was limited to swollen astrocytes as shown by electron microscopy. The addition of a potent inhibitor as determined by the tissue slice assay, i.e., one of the (indanyloxy)alkanoic acids, to the perfusate together with systemic pretreatment, totally blocked astroglial swelling and associated increases in tissue ion contents. This not only serves as an assay but confirms the validity of the *in vitro* slice swelling assay.[51]

C. Clinical Studies

To confirm the relevance of the animal assay to the human condition, it was necessary to conduct a clinical study. Since, as will be shown later, ethacrynic acid exhibits modest *in vitro* and *in vivo* effects, a controlled clinical study was carried out using this drug.[20,32,33,51] Highly restrictive criteria for inclusion in the study were established.[20,57–63] This excluded patients with

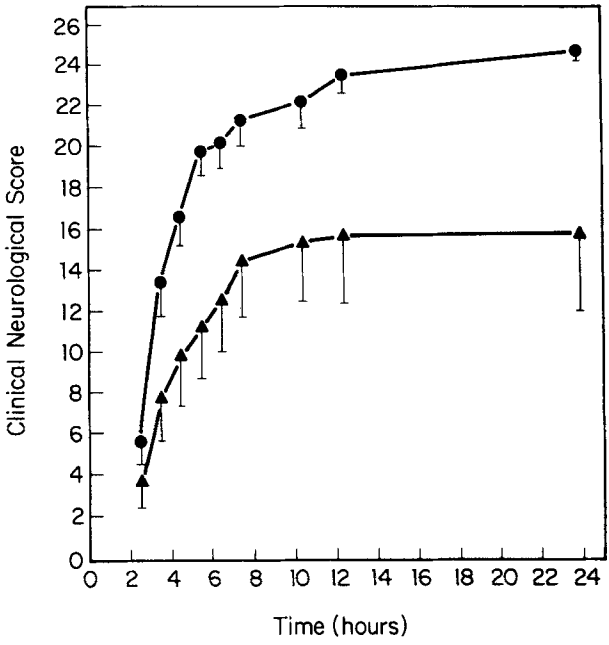


Figure 2. Clinical neurological score vs. time. Conditioned adult cats were anesthetized with a brief acting agent and subjected to a 1-min rapid acceleration/deceleration translational head injury followed 40 min later by a 60-min controlled period of hypoxemia (a pO_2 24 ± 1 torr), induced by ventilation (60 min) with a gas mixture of 6% O_2, balance N_2, followed by ventilation by room air. Survivors were scored from 2.5-h posttrauma with hypoxemia to the end of the experiment at 24 h at the times indicated. The clinical exam comprised 26 points accrued by sum of the scores for each of the following: arousal, reaction to pain, walking position, pupil reflex, cornea reflex, ear reflex, and respiratory pattern. Each plotted point represents mean values plus or minus standard errors for 12 or more determinations in control animals (▲) or animals treated with ethacrynic acid (●), 1 mg/kg iv, given slowly 10 min after the head injury and before the start of induced hypoxemia. The mean values for neurological score that were determined for treated animals differ significantly ($p < 0.025$) from comparable control values that were determined at 3.5 h and for each subsequent time plotted.

injuries other than head injury and included only those with a Glasgow Coma Score of 4 (which retrospectively anticipates a mortality rate of 50%).[57,64,65] These restrictive criteria limited the number of patients included in the study to 18 out of a possible 615 head-injured patients who entered the hospital during the 3.5 year study period.[51]

All patients received pulmonary management and standard medication with methylprednisolone sodium succinate and diphenylhydantoin sodium. Their blood volume and electrolyte levels were also rigidly controlled. Clinical neurological assessments were made hourly. Patients were alternately assigned to a control or ethacrynic acid treatment group. Patients assigned to the latter group received the standard care described above plus an initial loading dose of 50 mg of ethacrynic acid given within 1 to 4 h after arriving at the hospital. The loading dose was followed by a sustaining dose of 25 mg every 12 h, intravenously, for seven days.

Fluid balance was rigorously controlled in the control group of patients. For those patients in the ethacrynic acid treatment group, replacement fluids

rich in NaCl and KCl were administered, since these ions were lost by saluresis. In order to maintain normal blood volume and blood electrolyte levels, these patients were given a volume of normal saline equal to the previous hourly output plus an additional 10 mL of saline with supplemental K^+. The intent was to determine if ethacrynic acid beneficially influenced the clinical outcome from severe head injury *independent* of diuresis-related alterations in systemic solute and water balance. All patients received hourly clinical neurological assessments using the qualified Albany Head Injury Watch Sheet (AHIWS)[66] and Glasgow Coma Score.[67]

Of the 18 patients who met the stringent requirements, eight were arbitrarily assigned to the control group and ten to the etharcrynic acid treatment group. Eleven physiological and neurological parameters were measured for each patient during the critical 7–14 day period of intensive care following the head injury. These measurements included the duration of decerebration and coma, arterial pO_2 and pCO_2, blood pH and osmolality, serum Na^+ and K^+ levels, BUN, hematocrit, and urine output. No significant differences between the control and treatment groups were observed except the expected increase in urinary output of the treatment group. Despite the differences in urinary output, there were no significant differences in systemic fluid balance and electrolyte concentration. Therefore, any effect of the ethacrynic acid treatment could not be ascribed to diuretic induced hyperosmolality.

The data in Table II demonstrate that there were no significant differences in the control and treatment groups at the beginning of the study. The data included the time interval between the accident and arrival to the hospital (A), initial general neurological status (B and C), specific clinical brain-stem function (D), and findings on computerized axial tomography (E). However,

Table II.
Comparison of the Clinical Effects of Ethacrynic Acid Treatment with Untreated Controls

Label	Parameter	Control Patients	Treated Patients
		ENTRANCE DATA	
A	Avg. time (hrs.) from accident to arrival at hospital	1.9	1.6
B	Mean AHIWS score	5.1	5.4
C	Mean initial Glasgow score	4.0	4.0
D	Initial computerized axial tomograph of brain		
	No. with diffuse swelling	5	6
	No. with brain contusion	3	4
E	Initial oculovestibular/oculocephalic reflexes		
	No. of patients intact	6	6
	No. of patients impaired	2	4
		EXIT DATA	
F	No. of survivors	4	9
G	No. of deaths	4	1
H	No. of functional survivors	2	9
I	No. of nonfunctional survivors	2	0
J	No. of good results (H)	2	9
K	No. of poor results (G + I)	6	1
L	Rate of neurological recovery (AHIWS, units/day)	0 (± 0.23)	0.41 (± 0.33)

the outcome of the treated group was significantly better than the control group. This was true whether considered in terms of the number of survivors (F) ($p < 0.09$), functional survivors (H) ($p < 0.05$), good or poor results as defined by the Glasgow Outcome Scale (J and K) ($p < 0.01$), or rate of clinical neurological recovery as defined by the AHIWS (L) ($p < 0.05$). There was 10% mortality among the treated group (one of ten) compared to 50% (four of eight) among the control group. This latter value was predicted on the basis of the criterion by which all the patients were selected. It should be noted that two of the four survivors in the control group were non-functional (i.e., they were brain-dead); thus, there were only 25% *functional* survivors in the control group.

The data obtained in the *in vitro, in vivo,* and clinical studies appeared sufficient to establish the rationale for our approach to the development of agents for the treatment of traumatic brain injury and medical disorders with similar sequelae. Likewise, the assays appeared to be adequate to permit screening for leads, development of leads, and selection of clinical candidates. Finally, suggestive data on the role of chloride in astroglial swelling encouraged basic studies regarding the biochemistry of this phenomenon.

IV. IN VITRO SCREENING PROGRAM

Initially, members of distally acting and loop diuretics of the sulfonamide class were evaluated in the *in vitro* cerebrocortical slice assay. Some of the results are shown in Table III. Compounds of this class were generally inactive or only weakly active. Some exceptions were chloroaminophenamide, hydrochlorothiazide, and bumetanide. The reasons for these exceptions may become clearer as more is learned about the specific transport systems that are involved in astrocytic swelling.

The non-sulfonamide diuretics which were thought to operate in the thick ascending limb of Henle's loop appeared to be worthy of special attention since they were generally regarded as chloride transport inhibitors. Again, some perplexing anomalies appeared as seen in Table III. Ethacrynic acid was modestly active but its much less diuretic dihydro derivative was at least equipotent. MK-935, which is a more potent diuretic in dogs than ethacrynic acid, was inactive. MK-196 was as active as ethacrynic acid, but its less diuretic congener MK-473 was more active than either ethacrynic acid or MK-196. Interestingly, the inhibitory activity on cerebrocortical slice swelling appeared to lie in the more diuretic (+)-enantiomer of MK-473. The distally acting tienilic acid was inactive, but its loop-acting benzofuran congener (compound A) was moderately active and, again, the diuretic (+)-enantiomer appeared to be responsible for the observed inhibitory effect on cerebrocortical slice swelling.

As mentioned earlier, the I_{50} (concentration required for 50% inhibition) of a compound is only one determinant of the intrinsic activity of a given compound. A concentration versus inhibition curve provides important information regarding the therapeutic potential of the compound. Some compounds generate curves which never reach 100% inhibition, and others exhibit a very narrow range of complete inhibition. Many compounds produce V- or U-shaped curves with higher concentrations exhibiting no inhibition. This

Table III.
In Vitro Cerebrocortical Slice Assay of Known Diuretics

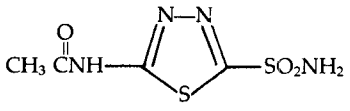
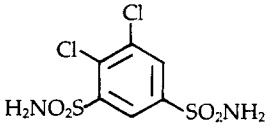
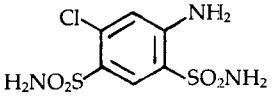
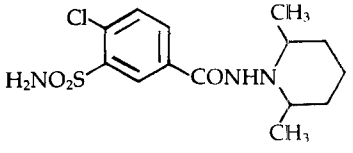
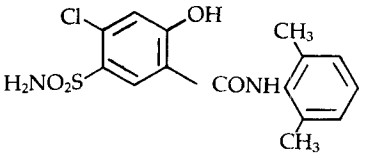
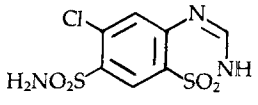
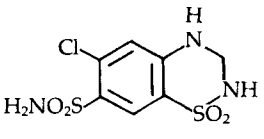
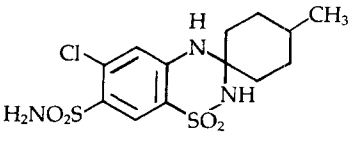
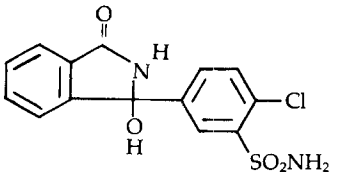
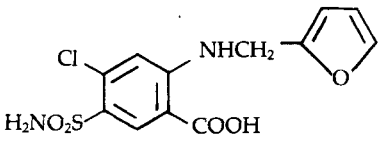
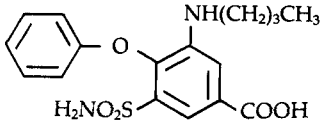
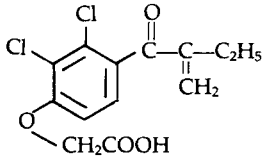
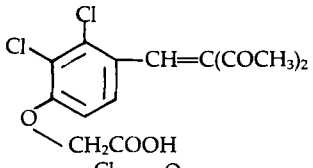
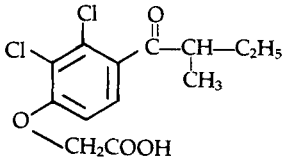
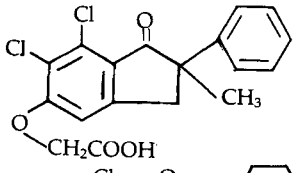
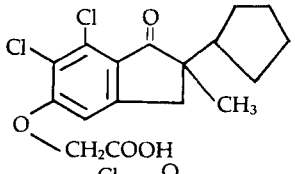
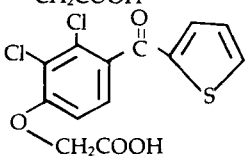
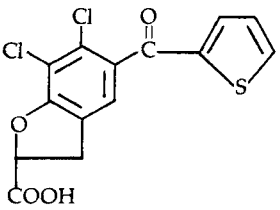
Name	Structure	I_{50} (Molar)
Acetazolamide		2×10^{-3}
Dichlorophenamide		Inactive*
Chloroaminophenamide		5×10^{-8}
Clopamide		Inactive*
Xipamide		Inactive*
Chlorothiazide		Inactive*
Hydrochlorothiazide		2×10^{-7}
MK-455		Inactive*
Chlorothalidone		Inactive*

Table III.
(Continued)

Name	Structure		I_{50} (Molar)
Furosemide			10^{-5}
Bumetanide			5×10^{-7}
Ethacrynic Acid			10^{-6}
MK-935			Inactive*
Dihydroethacrynic Acid			5×10^{-7}
MK-196			10^{-6}
MK-473		(±) (+) (−)	2×10^{-7} 10^{-7} Inactive**
Tienilic Acid			Inactive*
Compound A		(±) (+) (−)	4×10^{-7} 2×10^{-7} Inactive**

*No inhibitions at 10^{-6} M
**Inactive at 10^{-5} M

increase in swelling at the higher concentrations of a compound may be due to unwanted effects, such as the inhibition of the (Na^+,K^+) pump. In any event, both the I_{50} value and the shape and nature of the concentration/inhibition curve are used in making judgments regarding leads, as well as drug candidates. Ideally, a broad range of 100% or nearly 100% inhibition is sought and, if the curve is U-shaped, the drug-induced swelling occurs only at relatively high concentrations where its therapeutic consequences would not be a consideration.

V. THE DESIGN AND DEVELOPMENT OF BRAIN INJURY DRUGS

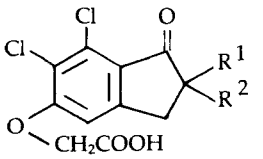
The *in vitro* cerebrocortical slice assay has served to identify a number of lead or prototype compounds for exploitation by a synthetic program. In addition, structure/activity studies within a chemical series have identified new subclasses of compounds that merited exploration as separate leads. As a result, a number of series have been explored in varying degrees of detail. Two series that were explored rather exhaustively will serve to illustrate our investigation.[68–76] These two series are the (aryloxy)alkanoic acids[51] and the [(1H-fluoren-7-yl)oxy]alkanoic acids.[70]

A. (Aryloxy)alkanoic Acids[51]

The screening of diuretics in the *in vitro* cerebrocortical slice assay afforded several attractive leads among which was MK-473 (see Table III). It is noteworthy that there was a marked chiral effect with the (+)-enantiomer [Table IV, A-2(+)] having an $I_{50} = 10^{-7}$ M, while the (−)-enantiomer [Table IV, A-2(−)] was inactive even at 10^{-5} M. Table IV demonstrates the effect of varying the two two-position substituents. Replacement of the 2-methyl group of A-2 by ethyl (A-3) increased the potency by 100-fold. Replacement by butyl (A-4) was nearly as effective. Here it can be seen that the (+)-enantiomer (A-4(+)] was five orders of magnitude more active than the (−)-enantiomer [A-4(−)]. Replacement of the 2-cyclopentyl group by benzyl (A-6) also increased potency appreciably.

Table V reveals the effect of varying both the 2-position substituents (R^1 and R^2) and the group (A) bridging the ether oxygen atom to the carboxyl group. Replacement of the methylene bridge of MK-473 (A-2) by two to six methylene units (A-9 to A-13) generally increased the potency with the peak increase occurring at $(CH_2)_3$ (A-10) and $(CH_2)_4$ (A-11). Where the enantiomers of these compounds were evaluated, the (+)-enantiomer [(A-10(+)] was somewhat more active than the (−)-enantiomer [A−10(−)] in the instance where A = $(CH_2)_3$, while the reverse was true with the compound where A = $(CH_2)_5$ [compare A-12(+) with A-12(−)]. The only other noteworthy effect was where R_2 = butyl and A = $(CH_2)_3$ (A-16). Here again the (−)-enantiomer [A-16(−)] was more active than the (+)-enantiomer [A-16(+)]. It is interesting to note that the more active (+)-enantiomer of compound A-10 has the same R absolute configuration at the 2-position as that of the more active (−)-enantiomer of compound A-16. A major objective in the design of these compounds was to attenuate their salidiuretic properties. That this goal was achieved with compounds displaying marked *in vitro* activity in the cerebrocortical slice inhibition assay is seen in Table V.

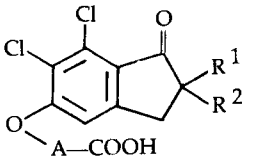
Table IV.
[(6,7-Dichloro-2,3-dihydro-1-oxo-2-substituted and 2,2-disubstituted- 1H-inden-5-yl)oxy]alkanoic Acids



No.	R^1	R^2	Enantiomer	% Distribution in Octanol vs. 7.4 Buffer[a]	pKa in 30% Ethanol[b]	Relative Saluretic Activity	In Vitro Assay I_{50} (M)[d]
A-1	c-C_5H_9	H	±	83	3.9	2	10^{-5}
A-2[e]	c-C_5H_9	CH_3	±	90	3.9	2	2×10^{-7}
A-2(+)[f]	c-C_5H_9	CH_3	+	90	3.9	3	10^{-7}
A-2(−)[f]	c-C_5H_9	CH_3	−	90	3.9	2	$>>10^{-5}$
A-3	c-C_5H_9	C_2H_5	±	97	3.9	2	10^{-9}
A-4	c-C_5H_9	$(CH_2)_3CH_3$	±	99	3.9	1	3×10^{-9}
A-4(+)[f]	c-C_5H_9	$(CH_2)_3CH_3$	+	99	3.9	1	3×10^{-10}
A-4(−)[f]	c-C_5H_9	$(CH_2)_3CH_3$	−	99	3.9	1	5×10^{-5}
A-5	CH_2-c-C_5H_9	H	±	97	3.8	1	10^{-8}
A-6	$CH_2C_6H_5$	CH_3	±	91	3.8	1	10^{-9}
A-7	C_6H_5	CH_3	±	84	3.7	4	10^{-6}
A-8	C_6H_5	C_6H_5	±	99	3.8	0	10^{-8}
	Ethacrynic Acid			61	3.5	4	10^{-6}
	Dihydroethacrynic Acid			96	3.5	±	5×10^{-7}
	Acetazolamide			22	7.2	1	2×10^{-3}
	Furosemide			28	4.5	4	10^{-5}

[a]Determined by the method of A. Albert and E.P. Serjeant, Eds., in *Ionization Constants of Acids and Bases*, Wiley, New York, 1962.
[b]Determined by the method of R.A. Scherrer and S.M. Howard, *J. Med. Chem.*, **20**, 53 (1977).
[c]The relative salidiuretic efficacies are scored in a fashion similar to those reported previously[47]: 0 = inactive; ± = marginal; 1 = weak; 2 = moderate; 3 = marked; 4 = very marked.
[d]These data were obtained as described previously.[51]
[e]This is MK-473, referred to as "DCPI" in ref. 45.
[f]X-ray analysis reveals A-2(+) and A-4(+) are R(+) and A-2(−) and A-4(−) are S(−).

Table V.
[(6,7-Dichloro-2,3-dihydro-1-oxo-2,3-disubstituted-1H-inden-5- yl)oxy]alkanoic Acids



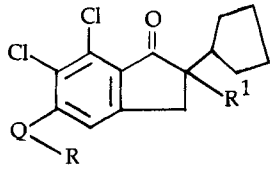
No.	R^1	R^2	A	Enantiomer	Relative Salidiuretic Activity	% Distribution in Octanol vs. 7.4 Buffer	pKa in 30% Ethanol	In Vitro[a] Assay I_{50} (M)
A-9	c-C_5H_9	CH_3	$(CH_2)_2$	±		99	4.6	2×10^{-8}
A-10	c-C_5H_9	CH_3	$(CH_2)_3$	±	±	99	5.7	2×10^{-8}
A-10(+)	c-C_5H_9	CH_3	$(CH_2)_3$	+	0	99	5.7	10^{-8}
A-10(−)	c-C_5H_9	CH_3	$(CH_2)_3$	−	±	99	5.7	2.5×10^{-7}
A-11	c-C_5H_9	CH_3	$(CH_2)_4$	±		99.5	5.7	10^{-8}
A-12	c-C_5H_9	CH_3	$(CH_2)_5$	±		99.5	6.1	10^{-7}
A-12(+)	c-C_5H_9	CH_3	$(CH_2)_5$	+		99.5	6.1	$>10^{-6}$
A-12(−)	c-C_5H_9	CH_3	$(CH_2)_5$	−		99.5	6.1	5×10^{-8}
A-13	c-C_5H_9	CH_3	$(CH_2)_6$	±		99.7	6.6	6×10^{-8}
A-14	c-C_5H_9	C_2H_5	$(CH_2)_3$	±	±	98.7	3.8	$>10^{-8}$
A-15	c-C_5H_9	$(CH_2)_2CH_3$	$(CH_2)_3$	±	±	99.6	4.1	8×10^{-9}
A-16	c-C_5H_9	$(CH_2)_3CH_3$	$(CH_2)_3$	±	0	99.8	4.4	4×10^{-9}
A-16(+)[c]	c-C_5H_9	$(CH_2)_3CH_3$	$(CH_2)_3$	±	0	99.9	4.4	10^{-6}
A-16(−)[c]	c-C_5H_9	$(CH_2)_3CH_3$	$(CH_2)_3$	−	0	99.9	4.4	2×10^{-9}
A-17	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	$(CH_2)_3$	±		99.9	6.1	10^{-6}
A-18	C_6H_5	CH_3	$(CH_2)_3$	±	±	99.9	6.4	2.5×10^{-7}
A-19	c-C_5H_9	CH_3	$C(CH_3)_2$	±		98.6	3.7	$>10^{-4}$
A-20	c-C_5H_9	CH_3	$C(CH_2)_3$	±		98.6	3.8	10^{-6}
A-21	c-C_5H_9	CH_3	$CH_2CH{=}CHCH_2$	±		98	5.1	$>4 \times 10^{-5}$
A-22	c-C_5H_9	CH_3	$CH_2C_6H_4$-p	±		100	6.2	$>10^{-5}$

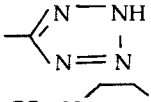
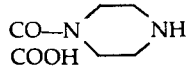
[a]These data were obtained as described previously.[51]
[b]See Note c in Table IV.
[c]X-ray analysis reveals that A-16(+) is R(+) and A-16(−) is S(−).

Table VI serves to demonstrate the effect of varying the bridge (Q) between the 5-position of the indane ring and the carbonyl group (R), as well as the effects of derivatizing the carboxy group. Compound A-10 and its ethyl ester (A-23) were equipotent, but the 1-carboxy-1-methylethyl ester (A-26) was considerably more potent. However, the 1-carboxy-1-methylethyl esters of A-2 (A-24) and A-4 (A-25) showed little change in activity over that of their parent acids.

The acidic N-mesyl amide of A-2 (A-27) was slightly more active, but that of A-4 (A-28) was less active than their corresponding carboxylic acids. Rather surprisingly, the tetrazole analog of A-2 (A-29) was devoid of activity at 10^{-6} M. The amide of A-2 derived from piperazine (A-30) was as active as the parent (A-2). The thio analogs of A-2 (A-31) and A-4 (A-32) were inactive. Replacement of the ether oxygen atom of A-2 with NH (A-33) produced a considerable increase in potency and the major biological effect lay in the

Table VI.
6,7-Dichloro-2-cyclopentyl-2,3-dihydro-2,5-disubstituted-1H-inden-1-ones



No.	R^1	Q	R	Enantiomer	In Vitro[a] Assay I_{50} (M)
A-23	CH_3	$O(CH_2)_3$	$COOC_2H_5$	±	10^{-8}
A-24	CH_3	OCH_2	$COOC(CH_3)_2COOH$	±	8×10^{-8}
A-25	$(CH_2)_3CH_3$	OCH_2	$COOC(CH_3)_2COOH$	±	2×10^{-8}
A-26	CH_3	$O(CH_2)_3$	$COOC(CH_3)_2COOH$	±	8×10^{-10}
A-27	CH_3	OCH_2	$CONHSO_2CH_3$	±	8×10^{-8}
A-28	$(CH_2)_3CH_3$	OCH_2	$CONHSO_2CH_3$	±	10^{-8}
A-29	CH_3	OCH_2		±	$>10^{-6}$
A-30	CH_3	OCH_2		±	10^{-8}
A-31	CH_3	SCH_2	COOH	±	$>10^{-7}$
A-32	CH_3	$S(CH_2)_3$	COOH	±	$>10^{-7}$
A-33[b]	CH_3	$NHCH_2$	COOH	±	8×10^{-9}
A-33(+)[b]	CH_3	$NHCH_2$	COOH	+	10^{-7}
A-33(−)[b]	CH_3	$NHCH_2$	COOH	−	4×10^{-9}
A-34[c]	CH_3	—	COOH	±	$>10^{-7}$
A-35[c]	CH_3	CH_2	COOH	±	$>>10^{-6}$
A-35(+)[c]	CH_3	CH_2	COOH	+	$>10^{-7}$
A-36[c]	$(CH_2)_3CH_3$	CH_2	COOH	±	$>>10^{-6}$
A-37[c]	CH_3	$(CH_2)_2$	COOH	±	$>10^{-6}$
A-38[c]	$(CH_2)_3CH_3$	$(CH_2)_2$	COOH	±	$>10^{-7}$
A-39[c]	CH_3	$(CH_2)_4$	COOH	±	2×10^{-8}

[a]These data were obtained as described previously.
[b]See Ref. 68.
[c]See Ref. 69.

Table VII.
In Vivo Activity of A-10 and A-10(+) in the Acceleration-Deceleration Head Injury Assay

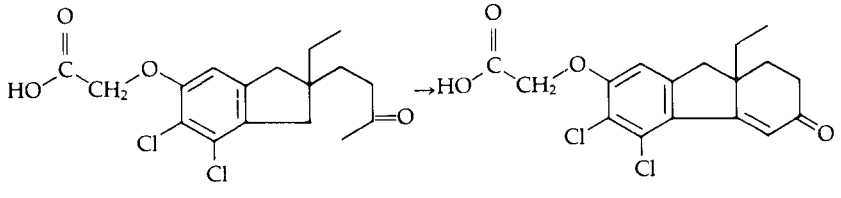
Compound No.	I.V. Dose mg/kg	Treated Animals Deaths/ Total	Treated Animals % Mortality	Control Animals Deaths/ Total	Control Animals % Mortality	%
A-10	5	4/16	25	38/75	51	25
A-10(+)	1	5/16	31	38/75	51	20

(−)-enantiomer [A-33(−)]. Deletion of the OCH_2 moiety of A-2 to give A-34 produced a loss in biological activity. Similar results were obtained by deletion of the ether oxygen atom of A-2 (A-35) or A-4 (A-36) or by replacing the ether oxygen atom with CH_2 for A-2 (A-37) or A-4 (A-38). However, replacement of the ether oxygen atom of A-10 by CH_2 gave a compound (A-39) whose potency was as great as A-10 itself.

One of the more active members of the non-diuretic (indanyloxy)alkanoic series was A-10 and its more potent enantiomer A-10(+). These compounds were evaluated in the *in vivo* acceleration-deceleration assay and the results are summarized in Table VII. At an intravenous dose of 5 mg/kg [i.e., containing 2.5 mg/kg of the active enantiomer A-10(+)], the mortality was 4 of 16 compared to 38 of 75 for the untreated controls for a $\Delta = 26\%$. When A-10(+) was administered at 1 mg/kg, the mortality was 5 of 16 for a $\Delta = 20\%$.

B. [(2,3,9,9a-Tetrahydro-3-oxo-9a-substituted-1H-fluoren-7-yl)oxy]alkanoic Acids

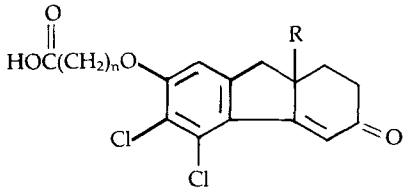
One of the (indanyloxy)alkanoic acid analogs, [2-ethyl-2-(3-oxobutyl)-6,7-dichloro-1-oxo-1,2-dihydro-1H-inden-5-yl)oxy]acetic acid (B-0), was cyclized to form the corresponding [(5,6-dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1H-fluoren-7-yl)oxy]acetic acid (B-2).[70]



B-O B-2

Inasmuch as this observation represented a structural modification of the diuretic (indanyloxy)acetic acids that virtually deleted salidiuretic activity, it was a candidate for evaluation in the *in vitro* cerebrocortical slice assay (see Table VIII). When B-2 was shown to exhibit relatively good intrinsic activity in inhibiting swelling as measured by this test, this compound became a prototype for a new series of antibrain-edema drugs. That this was a specific biological response was suggested by the marked chiral effect exhibited by the (+)-enantiomer [B-2(+)] which possessed a 3000-fold greater activity than the (−)-enantiomer [B-2(−)].

Table VIII.
[(5,6-Dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-substituted-IH-fluoren-7-yl)oxy]alkanoic Acids



No.	n	R	Enantiomer	% Distribution in Octanol vs. 7.4 Buffer	pKa in 30% Ethanol	In Vitro Assay[a] I_{50} (M)
B-1	1	$-CH_3$	±	62	3.75	10^{-8}
B-2	1	$-C_2H_5$	±	81	3.6	3×10^{-8}
B-2(+)[b]	1	$-C_2H_5$	+	81	3.6	10^{-8}
B-2(−)[b]	1	$-C_2H_5$	−	81	3.6	3×10^{-5}
B-3	1	$-(CH_2)_2CH_3$	±	93	3.6	4×10^{-11}
B-3(+)[b]	1	$-(CH_2)_2CH_3$	+	93	3.6	2×10^{-11}
B-3(−)[b]	1	$-(CH_2)_2CH_3$	−	93	3.6	10^{-5}
B-4	1	$-CH(CH_3)_2$	±	89	3.83	$>10^{-7}$
B-5	1	$-(CH_2)_3CH_3$	±	98	3.5	$>10^{-8}$
B-6	1	c-C_5H_9	±	97	3.83	3×10^{-9}
B-6(+)	1	c-C_5H_9	+	97	3.83	5×10^{-8}
B-6(−)	1	c-C_5H_9	−	97	3.83	5×10^{-10}
B-7	1	$-CH{=}CH_2$	±	70	3.5	$>10^{-8}$
B-8[c]	1	$-CH_2CH_2OH$	±	8.0	3.8	8×10^{-12}
B-9	3	$-C_2H_5$	±	99.2	3.7	$>10^{-5}$
B-10	3	$-(CH_2)_2CH_3$	±	99.8	3.7	3×10^{-9}

[a]These data were obtained as described previously[51].

[b]X-ray analyses of crystals of these compounds using Hamilton R-factor values revealed that B-2(+) and B-3(+) had the 9a-R absolute configuration; therefore, B-2(−) and B3(−) have the 9a-S absolute configuration.

[c]See Ref. 71.

1. *Structure/Activity Studies*

Table VIII demonstrates the effect of variation of the 9a-substituent (R) on the results observed in the *in vitro* cerebrocortical slice assay. The 9a-methyl compound, B-1, is as active as the lead compound B-2. Increasing the chain length to propyl (B-3) enhanced the potency by about 750-fold over the lead compound (B-2). Again, the marked chiral effect is seen with the (+)-enantiomer [B-3(+)] being twice as active as the racemate (B-3) and 500,000-fold more active than the (−)-enantiomer [B-3(−)]. The concentration/response curves of these two enantiomers shown in Figure 3 reveal more than a difference in potency. Not only are the curves not parallel, but the (−)-enantiomer never produced more than a 50% inhibition of swelling and concentrations above the I_{50} value produced a concentration-related incremental increase in swelling. The absolute configuration at the 9a-position for the active (+)-enantiomers B-2(+) and B-3(+) was R, while the less active enantiomers B-2(−) and B-3(−) had the S-configuration. As anticipated, both B-2(+) and B-3(+) exhibited markedly attenuated salidiuretic effects in dogs.

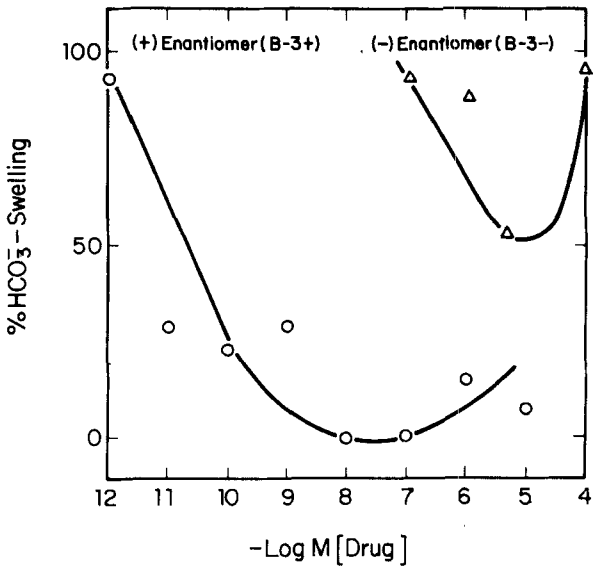


Figure 3. The *in vitro* concentration-response curve for B-3(+) and B-3(−) using the cat cerebrocortical slice assay.

From the *in vitro* data, it is postulated that a hydrophobic binding site exists above the plane of the hydrocarbon backbone that is optimally occupied by the methyl group of the 9a-propyl moiety of B-3(+) in its extended conformation. Molecular modeling of the other compounds in Table VIII revealed that only the 2-hydroxyethyl group of B-8 was as efficient as the *n*-propyl group in filling this region. Interestingly, as predicted, the cyclopentyl group of B-6(−) approached the efficiency of the *n*-propyl group and was quite superior to the isopropyl (B-4) and *n*-butyl groups (B-5).

The dramatic detrimental effect on activity by branching or increasing the chain length of the 9a-substituent (R) is demonstrated by the minimal *in vitro* activity of the isopropyl (B-4) and *n*-butyl (B-5) compounds at concentrations of 10^{-7} M and 10^{-8} M, respectively. As mentioned, the cyclopentyl compound (B-6) was quite active, but the (−)-enantiomer [B-6(−)] was the more potent one. Although substitution of a vinyl group (B-7) at the 9a-position was contraindicated, as predicted, the 9a-(2-hydroxyethyl) substituent (B-8) resulted in a very potent compound. Interestingly, this compound is markedly less lipophilic than its carbon isostere (B-3) as revealed by the difference in their octanol vs. pH 7.4 buffer distribution ratios.

Although increasing the side chain length from acetic acid to butyric acid in the (indanyloxy)alkanoic acid series (see A-2 vs. A-10) produced a beneficial effect on activity, this maneuver had the opposite effect on B-2 (to give B-9) and B-3 (to give B-10). None of the structural changes reported in Table VIII had a very profound effect on the acidity of the molecule; therefore, the ratio of ionized/unionized drug was similar in each instance.

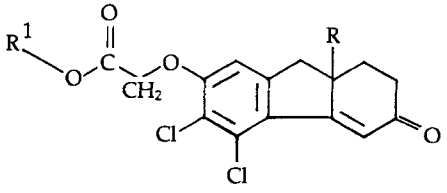
The effect of esterification on the biological activity of the carboxylic acids

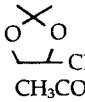
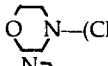
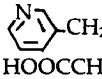
listed in Table VIII is shown in Table IX. Although conversion of B-1 to its ethyl ester (B-11) had no effect on its activity, conversion of B-2 to its ethyl ester (B-12) produced a 100-fold increase in potency. This effect carried over to the two enantiomers B-12(+) and B-12(−). The 3-hydroxypropyl ester (B-13) and the 2-oxopropyl ester (B-16) exhibited enhanced activity over that of B-2, but this was not observed with esters B-14 and B-15.

The relatively basic ester, B-17, exhibited little activity, while the more weakly basic ester (B-18) was 200 times more potent than the parent acid (B-2). Esters bearing a solubilizing carboxy function like the glycolic acid esters B-19 and B-19(+), enhanced potency somewhat, but their salts were rather labile to hydrolysis. The corresponding 1-carboxy-1-methylethyl esters B-20 and B-20(+) were not only more potent, but their salts were much more stable to hydrolysis.

Several esters of B-3(+) were prepared. The methyl ester, B-21, was 10-

Table IX.
[(5,6-Dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-substituted-lH-fluoren-7-yl)oxy]alkanoic Acid Esters



No.	R^1	R	Enantiomer	% Distribution in Octanol vs. 7.4 Buffer	pKa in 30% Ethanol	In Vitro Assay[a] I_{50} (M)
B-11	CH_3—	—C_2H_5	±	99.5	—	2×10^{-8}
B-12	C_2H_5—	—C_2H_5	±	>99.8	—	2×10^{-10}
B-12(+)	C_2H_5—	—C_2H_5	+	>99.9	—	10^{-10}
B-12(−)	C_2H_5—	—C_2H_5	−	>99.8	—	$>>10^{-5}$
B-13	$HO(CH_2)_3$—	—C_2H_5	±	99.3	—	7×10^{-10}
B-14	$HOCH_2$— 	—C_2H_5	±	99.7	—	10^{-6}
B-15		—C_2H_5	±	>99.9	—	10^{-8}
B-16	CH_3COCH_2—	—C_2H_5	±	>99.9	—	10^{-9}
B-17		—C_2H_5	±	98.6	5.1 (+H⁺)	$>10^{-8}$
B-18		—C_2H_5	±	>99.9	3.32 (+H⁺)	10^{-10}
B-19	$HOOCCH_2$—	—C_2H_5	±	83	3.80	6×10^{-9}
B-19(+)	$HOOCCH_2$—	—C_2H_5	+	83	3.80	3×10^{-9}
B-20	$HOOCC(CH_3)_2$—	—C_2H_5	±	96	3.80	10^{-9}
B-20(+)	$HOOCC(CH_3)_2$—	—C_2H_5	+	96	3.80	5×10^{-10}
B-21	CH_3—	—$(CH_2)_2CH_3$	±	98	3.80	2×10^{-12}
B-22(+)	$HOOCC(CH_3)_2$—	—$(CH_2)_2CH_3$	+	98	3.80	4×10^{-12}
B-23(+)	$HOOCC(C_2H_5)_2$—	—$(CH_2)_2CH_3$	+	>99.9	3.40	4×10^{-11}
B-24(+)	$(CH_3)_2NCH_2CH_2$—	—$(CH_2)_2CH_3$	+	98.7	7.42 (+H⁺)	10^{-8}

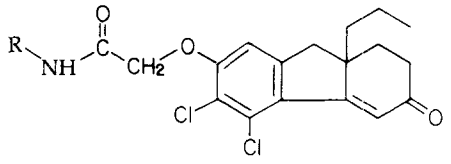
[a]These data were obtained as described previously.[51]

fold more potent than the parent acid and the 1-carboxy-1-methylethyl ester [B-22(+)] was slightly more active. However, the homologous 1-carboxy-1-ethylpropyl ester B-23(+), showed no enhancement of activity and the basic ester [B-24(+)] was considerably less active than the parent acid.

Table X lists the amide and several substituted amides of B-3 and B-3(+) were prepared and evaluated in the *in vitro* assay. The amide [B-25(+)], the basic amide (B-26), and the 2,2-dimethylhydrazide (B-27) displayed markedly reduced activity as compared to the parent acid. The methanesulfonamide [B-28(+)] and the ethanesulfonamide [B-29(+)] exhibited weakly acidic character but displayed considerably attenuated activity in comparison to B-3(+). However, the amide derived from glycine B-30(+) was somewhat more active than B-3(+) and gave an *in vitro* concentration/response curve which was parallel to that of B-3(+) shown in Figure 3. As will be discussed later, B-30(+) is more hydrophilic and less acidic, i.e., less ionized at physiological pH than B-3(+).

Table XI and Figure 4 list a number of structural variations of the lead compounds B-2 or B-3. Substitution of a methyl group in the 4-position (C) of B-3 caused a marked reduction in activity. Annulation of the 9a-propyl group of B-3 to the 2-position to give D, produced a 2000-fold decrease in activity. Although replacement of the carboxy group by a 5-tetrazolyl moiety is often an acceptable maneuver, this congener (E) of B-2 possessed little activity. Replacement of the ether oxygen by sulfur in ethacrynic acid[77] or [[7-chloro-3-(2-fluorophenyl)benzisoxazol-6-yl]oxy]acetic acid[78] produced little decrease in the salidiuretic potency of these highly active loop diuretics. However, a similar tactic with B-3 to give F produced a nearly complete loss in activity. The phenol derivative (G) of B-3(+) was inactive. Introduction of a 1,2-double bond in B-2 to give H produced a marked reduction in activity. The oxime (I) derived from B-3(+) was about 150-fold less active than its parent.

Table X.
[(5,6-Dichloro-2,3,9,9a-tetrahydro-3-oxo-9a-substituted-1H-fluoren-7-yl)oxy]acetamides



No.	R	Enantiomer	% Distribution in Octanol vs. 7.4 Buffer	pKa in 30% Ethanol	In Vitro Assay[a] I_{50} (M)
B-25(+)	H—	+	99.3	—	$>10^{-7}$
B-26	$(CH_3)_2N(CH_2)_2$—	±	99.7	7.7 ($+H^+$)	$>10^{-6}$
B-27	$(CH_3)_2N$—	±	99.7	3.9 ($+H^+$)	$>10^{-7}$
B-28(+)	CH_3SO_2—	+	97	4.25	2×10^{-8}
B-29(+)	$C_2H_5SO_2$—	+	98.7	4.10	10^{-8}
B-30(+)[b]	$HOOCCH_2$—	+	88	4.35	5×10^{-12}

[a]These data were obtained as described previously.[51]
[b]See Ref. 74.

Table XI.
[(5,6-Substituted-2,3,9,9a-tetrahydro-3-oxo-9a-substituted-1H-fluoren-7-yl)oxy]acetic Acid Analogs

No.	Enantiomer	% Distribution in Octanol/ 7.4 Buffer	pKa in 30% Ethanol	In Vitro Assay[a] I_{50} (M)
C[b]	±	98	3.50	$>10^{-5}$
D[c]	±	85	3.50	8×10^{-8}
E[b]	±	93	3.90	$>10^{-7}$
F[b]	±	99	4.70	$>10^{-6}$
G[b]	+	11	6.18	$>10^{-5}$
H[b]	±	79	3.70	$>10^{-8}$
I[d]	+	95	3.80	3×10^{-9}
J-1[e]	± (α-diasteriomer)	81	4.03	$>10^{-6}$
J-2[e]	± (β-diasteriomer)	82	3.93	10^{-8}
K-1[b]	±	49	4.10	5×10^{-8}
K-2[b]	±	49	4.30	10^{-7}
L[b]	±	88	3.84	10^{-7}
M[b]	±	95	4.10	10^{-7}
N[f]	+	98	9.23 ($+H^+$)	2×10^{-12}

[a]These data were obtained as described previously.[51]
[b]See Ref. 70.
[c]See Ref. 73.
[d]See Ref. 74.
[e]See Refs. 72 and 75.
[f]See Ref. 76.

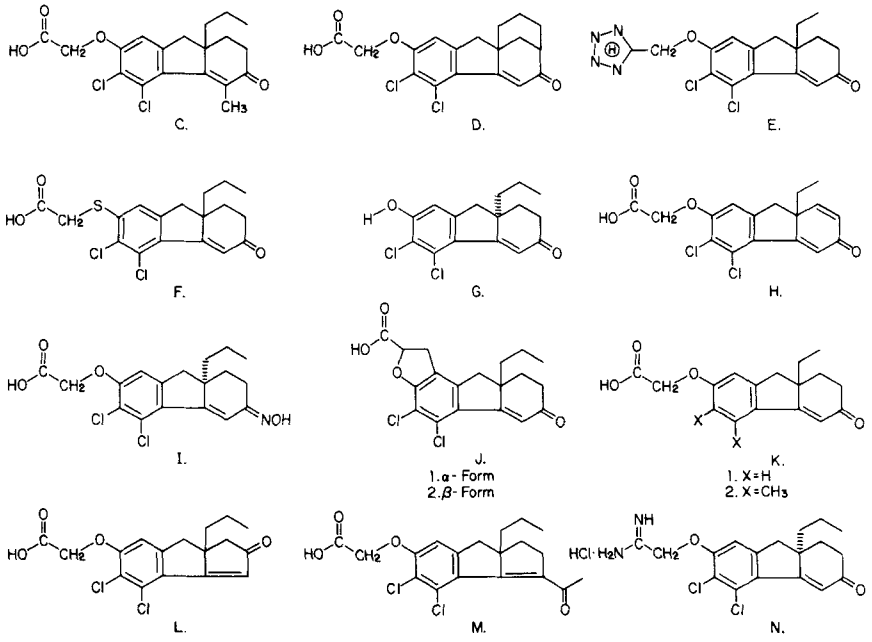


Figure 4. The structures of the analogs and congeners of the [(5,6-substituted-1,2,9,9a-tetrahydro-3-oxo-9a-substituted-1H-fluoren-7-yl) oxy]acetic acids.

The conversion of tienilic acid to the corresponding benzofuran, 6,7-dichloro-5-(2-thienylcarbonyl)-2-benzofurancarboxylic acid,[79] or annulation of MK-196 to the corresponding diasteriomeric 4,5-dichloro-7-methyl-6-oxy-7-phenyl-1,2,7,8-tetrahydro-6H-indeno[5,4-b]furan-2-carboxylic[80] acids produced a profound increase in salidiuretic activity. However, a similar manipulation of B-3 to give J-1 and J-2 produced a marked decrease in activity.

Replacement of the chloro groups of ethacrynic acid by methyl[81] had little effect on the salidiuretic activity, but replacement by hydrogen[81] nearly deleted activity. A similar maneuver with B-3 wherein the chloro groups were replaced by H- (K-1) or CH_3- (K-2) each produced a marked reduction both in *in vitro* activity and lipophilicity along with some decrease in acidity.

Two compounds were synthesized in which the cyclohexanone ring of B-3 was replaced by a five-membered ring. Removal of the 1-methylene group of B-3 to give compound L resulted in a 2500-fold loss in activity. A similar loss in activity was observed by a homolog in which the carbonyl group was exocyclic (M). Interestingly, the amidine analog (N) of B-3(+) displayed a 10-fold greater activity in the *in vitro* cerebrocortical slice assay and gave a concentration/response curve parallel to that seen for B-3(+) in Figure 3. Some other comparisons of N with B-3(+) will be discussed later.

2. *Species Specificity*

The early discovery of the potent *in vitro* inhibitory activity in the cat cerebrocortical slice assay in many compounds from the series of [(2,3,9,9a-tetrahydro-3-oxo-9a-substituted--1H-fluoren-7-yl)oxy]alkanoic acids and their derivatives suggested that selected members be evaluated in greater detail. The subsequent discovery of the marked potency of B-3(+) in the cat cerebrocortical slice assay presented the question of species response both to chloride transport inhibitors and to the structural class represented by B-3(+).

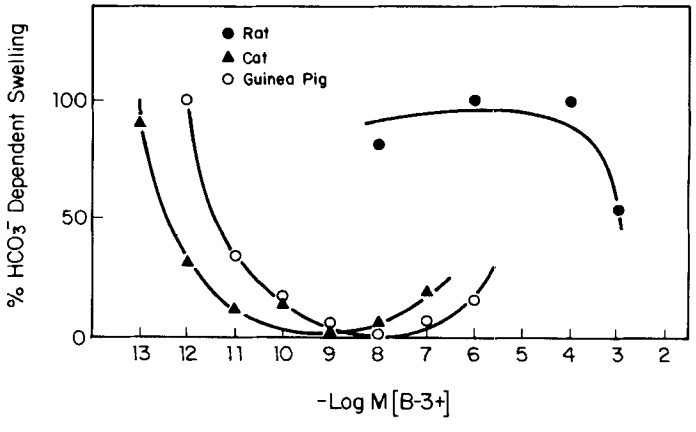


Figure 5. Concentration-response curve for inhibition by the compound B-3(+) of HCO_3^--dependent swelling of cerebrocortical slices from cat, guinea pig, and rat. The assays were similar for all species except for modifications in cutting the slices and removal of excess fluid due to the greater fragility of rat and especially guinea pig slices.

Figure 5 reveals that while inhibition of HCO_3^--dependent swelling was almost equally sensitive to inhibition by B-3(+) in both cat and guinea pig brain slices, those from the rat brain were quite insensitive to this agent. Guinea pig brain slices were about 10-fold less sensitive than cat brain slices ($I_{50} = 5 \times 10^{-13}$ vs. 5×10^{-12} M) while rat brain slices were about nine orders of magnitude less sensitive than cat brain slices.

There was also a remarkable species specificity in regard to responsiveness of cerebrocortical slice swelling and ion uptake inhibition by B-3(+). Data for the swelling and associated changes in ion content of the slices from these three species are seen in Table XII where both stimulation due to HCO_3^- and inhibition of such stimulation by varying concentrations of drugs are docu-

Table XII.
The K^+-stimulated, HCO_3^- -Dependent Swelling and Ion Uptake in Cerebrocortical Slices and Their Inhibition by B-3(+)

Media	% swelling	Cl^-	Na^+	K^+	n
		Cat			
27 mM K^+ HEPES	17.9 ± 1.0	87.4 ± 1.1	80.2 ± 1.5	84.5 ± 1.4	8
27 mM K^+ HEPES + 5 mM HCO_3^-, B-3(+), M	28.3 ± 1.1[c]	98.0 ± 1.0[c]	102.3 ± 2.7[c]	85.8 ± 1.4	5
10^{-7}	20.0 ± 2.1[e]	86.9 ± 2.9[e]	86.0 ± 2.3[e]	83.9 ± 3.3	4
10^{-8}	18.6 ± 2.9[d]	89.1 ± 2.1[e]	87.2 ± 2.3[a,e]	83.8 ± 3.2	4
10^{-9}	18.3 ± 0.9[f]	89.3 ± 2.7[e]	85.6 ± 2.2[a,f]	86.0 ± 1.0	6
10^{-10}	19.5 ± 1.9[e]	91.9 ± 2.5	86.9 ± 2.1[a,e]	86.9 ± 0.9	5
10^{-11}	19.1 ± 1.7[e]	87.9 ± 3.1[d]	86.4 ± 3.0[e]	88.8 ± 1.7	4
10^{-12}	21.3 ± 0.8[a,e]	90.5 ± 3.5	96.4 ± 2.2[c]	90.9 ± 0.8[b,d]	4
10^{-13}	27.3 ± 1.2[c]	98.5 ± 3.0[b]	107.3 ± 2.4[c]	87.6 ± 1.9	4
		Guinea pig			
27 mM K^+ HEPES	26.1 ± 0.8	107.7 ± 1.7	110.4 ± 1.6	86.7 ± 1.4	27
27 mM K^+ HEPES + 5 mM HCO_3^-, B-3(+), M	30.6 ± 1.0[c]	118.7 ± 2.5[c]	128.2 ± 2.4[c]	94.4 ± 2.1[b]	23
10^{-6}	26.8 ± 1.2[d]	103.8 ± 2.5[f]	115.0 ± 3.2[e]	95.0 ± 3.1[a]	8
10^{-7}	26.4 ± 0.9[d]	115.4 ± 1.8[b]	125.4 ± 1.6[c]	88.1 ± 1.8[d]	16
10^{-8}	24.4 ± 2.8[d]	103.8 ± 3.6[e]	108.6 ± 3.4[f]	90.8 ± 3.3	5
10^{-9}	26.2 ± 1.0[e]	105.2 ± 0.9[f]	110.0 ± 2.1[f]	95.9 ± 2.0[c]	4
10^{-10}	26.8 ± 1.7	101.4 ± 3.1[f]	108.3 ± 3.6[e]	101.3 ± 2.2[c]	8
10^{-11}	27.6 ± 0.9	94.8 ± 3.2	103.4 ± 2.4	88.9 ± 2.9	5
10^{-12}	30.9 ± 1.2[c]	100.5 ± 2.5	104.7 ± 3.0	87.7 ± 1.9	8
		Rat			
27 mM K^+ HEPES	15.6 ± 1.1	92.9 ± 1.5	101.5 ± 1.4	77.2 ± 1.5	18
27 mM K^+ HEPES + 5 mM HCO_3^-,B-3(+), M	22.1 ± 1.1[c]	104.7 ± 1.5[c]	123.1 ± 2.2[c]	76.7 ± 1.9	15
10^{-3}	19.1 ± 1.8	106.3 ± 1.2[c]	132.4 ± 4.4[c]	60.2 ± 1.7[c,f]	6
10^{-4}	22.8 ± 2.7[b]	92.3 ± 2.2[f]	112.1 ± 2.6[b,e]	73.7 ± 1.7	6
10^{-6}	22.7 ± 0.9[c]	101.5 ± 2.3[b]	127.0 ± 3.6[c]	71.9 ± 2.9	5
10^{-8}	20.9 ± 1.2[b]	101.4 ± 2.1[b]	118.9 ± 3.7[c]	75.4 ± 3.1	6

The details of this assay have been describd.[70] All data are mean values ± SEM. Drugs were always added in the presence of HCO_3^-. The drugs had no effect in the absence of added HCO_3^- (see Ref. 43). Key: *a* = $p<0.025$ vs. 27 *K*; *b* = $p<0.005$ vs. 27 K; *c* = $p<0.0005$ vs. 27 K; *d* = $p<0.025$ vs. 27 K + HCO_3^-; *e* = $p<0.005$ vs. 27 K + HCO_3^-; *f* = $p<0.0005$ vs. 27 K + HCO_3^-. All statistical values were determined by the two-tailed student's *t*-test.

mented. The data for rat slices reveal that inhibition of swelling at 10^{-3}M B-3(+) is associated with a decrease in K^+ and an increase in Na^+ content, suggesting inhibition of the (Na^+, K^+)-pump.

The lack of sensitivity to B-3(+) of the swelling and ion uptake of rat cerebrocortical slices was also observed when unidirectional influx and steady state levels of $^{36}Cl^-$ were measured in primary rat astrocyte cultures. However, these assays were conducted under conditions of normal K^+ concentration (i.e., 4.5 millimolar) in HCO_3^--buffered media and thus do not represent the high K^+-stimulated swelling of brain slices that is associated with astroglial swelling.[53] Previous studies presented possible stimuli for such edema,[44,52–54] which include release of transmitters and lactic acid, as well as a high CO_2/HCO_3^- value, either as single factor or as a combination of more than one. It seems more plausible that the compounds described in this study inhibit ion-transport mechanisms activated by such conditions rather than reacting directly with such effectors.

3. *Mechanism of Action Studies*

There are several experiments which might cast some light on both the mechanism of action of the agents described in these studies and the marked species differences observed. First, examination of guinea pig primary astrocyte cultures to determine whether "resting" unidirectional $^{36}Cl^-$ influx in these cells is more sensitive to inhibition than those of the rat is suggested. Second, determination should be made as to whether $^{36}Cl^-$ influx stimulated by transmitters, lactate, or high K^+ concentration is sensitive to inhibition in guinea pig and in rat primary cultures. Finally, the comparison of the inhibition of swelling in human cerebrocortical slices with those from the cat, guinea pig, and rat is a key experiment.

The rationale for selecting loop diuretics, such as furosemide and ethacrynic acid, initially for evaluation in the various brain injury assays was the evidence that astroglial swelling involved a chloride mediated process and that loop diuretics functioned by inhibiting such a mechanism. It was originally suggested that astroglial swelling induced in cat brain slices was due to the coupled effects of Cl^-/HCO_3^- and Na^+/H^+ exchange on ion transport in the astrocyte.[82,83]

Since the relevant ion transport systems are present in cat brain slices and primary astrocyte cultures[44,54,84] and because of the strategic location of the astrocyte processes around capillaries and neurons,[40,44,52,54,84] such swelling could have profound effects on neuronal function by exacerbating eschemia due to partial occlusion of blood flow and/or increase in the oxygen diffusion path length.[33b] The astrocyte uptake of Na^+ will decrease the inwardly directed Na^+-gradient and decrease the capacity of astrocytes to take up and terminate the action of released transmitters, such as amino acids,[70] catecholamines, and serotonin.[52] Since astrocytic swelling is a major component of cellular edema,[52] agents that inhibit this phenomenon and maintain normal transmembrane ion gradients in the early evolving posttrauma state could have a marked beneficial effect and constitute a new approach to the therapy of traumatic brain injury due to a variety of etiologies.

4. Intravenous In Vivo Studies with B-3(+)

Compound B-3(+) exhibited marked inhibitory activity in the cat cerebrocortical slice assay, apparently due to its potent effect on the Cl^-/HCO_3^- exchange system. Its lack of salidiuretic activity appears to be due to its ineffectiveness on the $Na^+ + K^+ + 2Cl^-$-cotransport system. This suggested a more extensive evaluation of this compound.

Table XIII records the effects of the intravenous administration of B-3(+) in the cat acceleration-deceleration head injury assay. There was a dose-related decrease in mortality of the drug-treated animals compared to the paired untreated controls as the dose was increased from 1 to 2.5 to 5 to 10 mg/kg. This was not only the most effective therapy that we have evaluated in this model of traumatic head injury, but there were other features of the compound that proved attractive. Not only were positive results achieved in the pharmacological and biochemical realm, but also at the anatomical level. Examination of the consequences of trauma on neurons and the therapeutic effects of B-3(+) on them requires a consideration of the major importance of the transport systems operating in astroglia, and it was demonstrated that such systems existed in primary rat astrocyte cultures.[82–85,87]

Recently, a study[86] was conducted to discover the qualitative and quantitative differences between the mechanism of ion transport inhibition characteristic of the classic loop diuretics and our structurally modified compounds which inhibited brain slice swelling and showed beneficial effects in experimental brain injury. We were especially interested in identifying the structural characteristics that were associated with the salidiuresis typifying loop diuretics in contrast to those peculiar to the inhibitors of astroglial swelling.

Table XIV summarizes the effects of some classic loop diuretics with representatives of our cerebrocortical slice inhibitors in an erythrocyte ion transport system, which discriminates between the $Na^+ + K^+ + 2Cl^-$-cotransport system and the DIDS-sensitive anion carriers ($LiCO_3^-$ influx) involving Cl^-/HCO_3^- exchange systems. It can be seen that he loop diuretics—bume-

Table XIII.
Activity of B-3(+) in the Cat Acceleration-Deceleration Head Injury Assay[a,d]

Control Animals		Treated Animals			
Deaths/ Total	% Mortality	Dose mg/kg	Deaths/ Total	% Mortality	Δ %[e]
14/25	56	1	12/25	48	8
8/19	42	2.5	5/21	24	18
7/22	32	5	1/19	5	27[b]
15/19	79	10	6/16	37.5	41.5[c]

[a]This assay was carried out as described previously.[51,70]

[b]Level of significance by Chi-square analysis: $p = <0.05$.

[c]Level of significance by Chi-square analysis: $p = <0.025$.

[d]For each study, pairs were matched according to sex and run simultaneously with one animal randomly chosen to receive the intravenously administered drug while the other animal was used as the control. Deaths were recorded over a period of 24 h following trauma, excluding the animals that died prior to application of hypoxia.

[e]Δ % refers to the difference in percent mortality between the treated animals and controls.

Table XIV.
Comparison of the Transport Inhibitory Effects of Compounds in the Dog Kidney, Cat Brain, and Human Erythrocyte[86]

Compound	Salidiuretic Activity[47]	In Vitro Cat Cerebrocortical Slice Assay[51] I_{50} (M)	Erythroctye I_{50} (M)	
			Outward $Na^+ + K^+ + 2Cl^-$ Cotransport	$LiCO_3^-$ Influx (Cl^-/HCO_3^- inhibition)
Bumetanide	+6	5×10^{-7}	6×10^{-7}	3×10^{-4}
Furosemide	+5	10^{-5}	2×10^{-5}	2×10^{-4}
Ethacrynic Acid	+5	10^{-6}	3×10^{-5}	2×10^{-4}
MK-473 (A-2)	+3	2×10^{-7}	10^{-4}	4×10^{-5}
A-4(+)	+1	3×10^{-10}	inactive	10^{-5}
A-10	±	3×10^{-10}	inactive	8×10^{-5}
A-16(−)	0	2×10^{-9}	inactive	10^{-5}
B-2(+)	0	10^{-8}	10^{-3}	8×10^{-7}
B-3(+)	±	2×10^{-11}	10^{-3}	8×10^{-8}
B-3(−)	0	10^{-5}	4×10^{-4}	4×10^{-5}

tanide, furosemide, and ethacrynic acid are effective co-transport inhibitors with generally modest and rather similar inhibitory effects on Cl^-/HCO_3^- exchange ($LiCO_3^-$ influx). Furthermore, the relative loop diuretic potency of these compounds roughly parallels their co-transport inhibitory activity. Moreover, these compounds have quite modest inhibitory effects in the *in vitro* cat cerebrocortical slice assay.

MK-473, which possesses modest loop diuretic activity, is a weaker co-transport inhibitor, but shows somewhat greater Cl^-/HCO_3^- exchange and cat cerebrocortical slice inhibitory activity.

The potent cerebrocortical slice inhibitory compounds of the (indanyloxy)alkanoic acid class (A-4(+), A-10, and A-16) and the [(2,3,9,9a-tetrahydro-3-oxo-1H-fluoren-7-yl)-oxy]acetic acid class [B-2(+) and B-3(+)] exhibit increased inhibitory activity against the Cl^-/HCO_3^- exchange system with markedly decreased activity against the co-transport system. This is especially noteworthy with B-3(+), where the contrasting effects of the enantiomeric B-3(−) on the cerebrocortical slice assay are reflected in the results in the study in erythrocytes.

5. *Electron Micrographic Studies*

Electron micrographs demonstrate that perivascular astrocytic swelling occurs in the cat acceleration-deceleration model of traumatic brain injury[55,70] and we have attributed the HCO_3^--dependent component of the swelling observed *in vitro* to edema of the same cells. This type of swelling is illustrated in Figure 6 where each of the three panels shows a brain capillary surrounded by neuropil. Capillary lumens are present approximately in the center of each of the three panels of Figure 6. Of particular note is the preparation from the nontraumatized animal in Figure 6A compared with that of the traumatized animal in Figure 6B, which has been perfuse-fixed 40 min after trauma. In Figure 6B, the perivascular astroglial processes are markedly swollen. Panel C illustrates a typical animal which was treated intravenously with B-3(+) 20

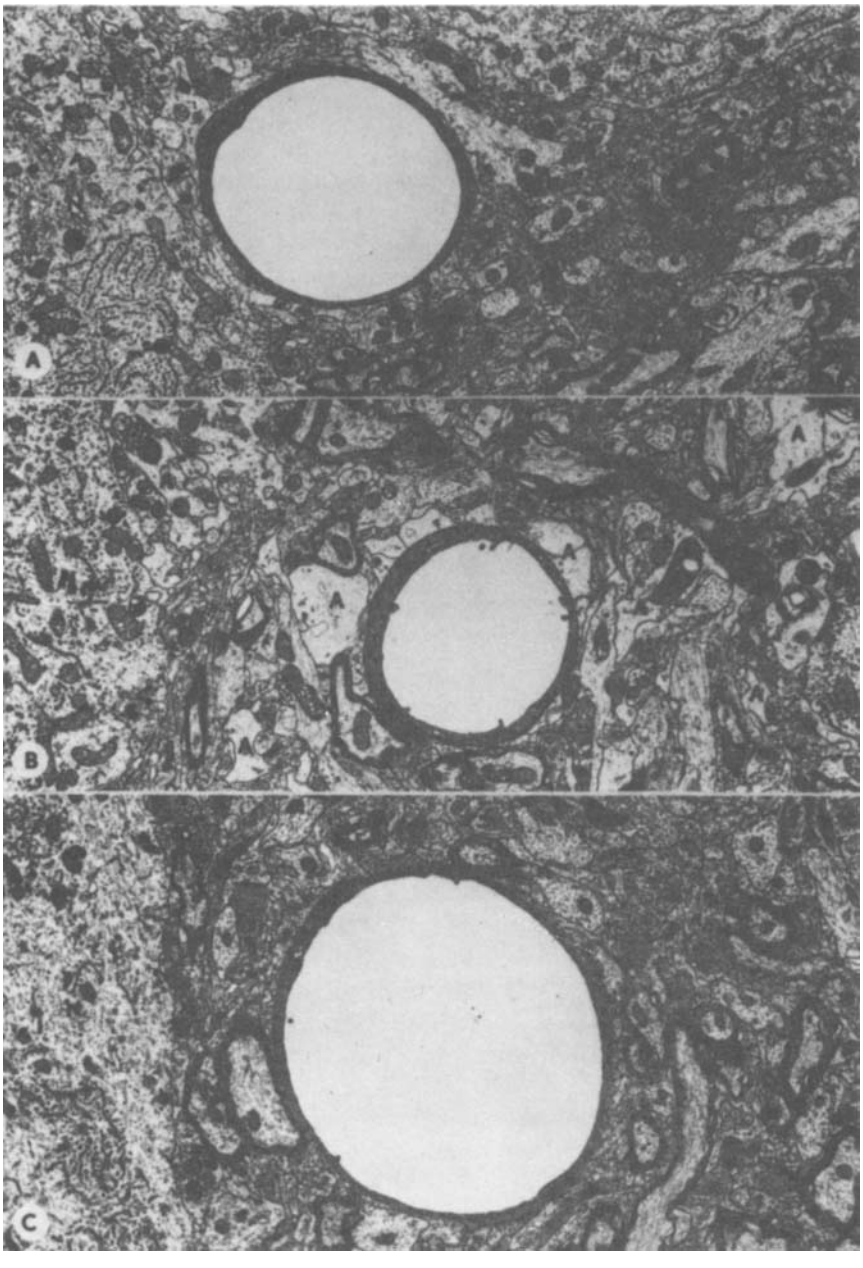


Figure 6. The effect of B-3(+) on the trauma induced astroglial swelling in the cat. The three figures were derived from the Betz cell layer of feline sensorimotor cortex. Panel A: Normal animals. Note the compact pericapillary neuropil. Neuronal cytoplasm at N. 8590×. Panel B: Animal killed 40 min after acceleration-deceleration injury. Note electron-lucent, expanded astrocytic profiles, some of which are labelled A. Neuron at N. 8590×. Panel C: Animal injected with B-3(+) at 10 mg/kg 20 min after shake injury and killed 20 min later. Astrocytic edema has been prevented and the neuropil resembles that seen in A. 8590×. Animals were perfused fixed and the brain tissue prepared for electron microscopy as previously described.[45]

min after the acceleration-deceleration event and then perfuse-fixed 20 min later. Here the perivascular region and the remainder of the neuropil are unswollen and appear normal.

Quantitative morphometric analysis of a number of animals further supported this qualitative evaluation.[88] Thus, this agent abolishes the anatomical evidence of the pathological response seen in traumatized but untreated animals.

6. The Problem of Drug Penetration of the Blood Brain Barrier (BBB)

The design and development of B-3(+) achieved the desired attenuation of salidiuretic activity and marked enhancement of the intrinsic antibrain edema effect. Furthermore, these properties could be explained, at least partially, by their decreased inhibitory effect on the $Na^+ + K^+ + 2Cl^-$-cotransport by system and markedly enhanced inhibitory activity on the Cl^-/HCO_3^- exchange system. However, at least one perplexing problem remained: Although B-3(+) possessed 50,000-fold the intrinsic potency of ethacrynic acid as measured by the cerebrocortical slice assay (see Tables IV and VIII), it required 2.5-fold the intravenous dose to achieve a comparable reduction in mortality in the acceleration-deceleration brain injury assay (compare Table I with Table XIII). There are several possible explanations for this observation including (1) differences in penetration rate of the blood brain barrier, (2) protein binding, (3) drug metabolism, etc.

Evidence exists to support the involvement of both of the first two factors. Although intravenous doses of B-3(+) in the range of 5 mg/kg exhibited a decrease in mortality in the acceleration-deceleration assay in cats, that dose of a ^{14}C-labelled form of the drug was not detectable by radioactivity in the brains of these animals.[89] It appears that the drug was crossing the blood brain barrier and accumulating in pharmacologically effective concentrations since Table XIII shows that there is a dose-related pharmacological response. However, the concentrations appearing in the brain were below the minimum level of detection of ^{14}C, which further emphasizes the high intrinsic activity of B-3(+) and low penetration of the blood brain barrier (BBB).

A protein-binding assay of B-3(+) with 4×10^{-4}M bovine serum albumin reveals that only 1.3% remains unbound.[90,91] Thus, there are at least two reasons for requiring higher than predicted *in vivo* doses of B-3(+).

7. Intracisternal In Vitro Studies with B-3(+)

To further elucidate the problem that B-3(+) has in penetrating the blood brain barrier, a study was conducted using the cat acceleration-deceleration assay in which B-3(+) was administered intracisternally.[92,93] The data from this study are presented in Table XV where it can be seen that an intracisternal dose of 57 μg/kg produced a 40% decrease in mortality over that of the matched control animals. This is approximately the same response that was observed using 10 mg/kg given intravenously (as shown in Table XIII). Thus, the intracisternal dose requirement is about 175-fold less than that of the intravenous route.

Table XV.
The Results of B-3(+) Injected Intracisternally on the Survival of Cats Subjected to Acceleration-Deceleration Plus Hypoxia Injury[92,93]

	Drug Treated Animals		Control Animals		Δ % (% Control Mortality Minus % Drug-Treat Mortality)
Intracisternal Dose (μg/kg)	Deaths/Total Animals	Percent Mortality	Deaths/Total Animals	Percent Mortality	
0.57	7/15	47	9/18	50	3
57	4/19	21	11/18	61	40

The second and third columns reveal the effects of the two doses of drug, and the fourth and fifth columns list the results from paired control animals injected with vehicle only. The experiments were run as pairs of drug-treated and control animals and the animal selected for each category was selected by a coin toss. Level of significance = $p < 0.025$ by Chi-square analysis as compared to control results.

VI. DESIGNING DRUGS TO CROSS THE BBB

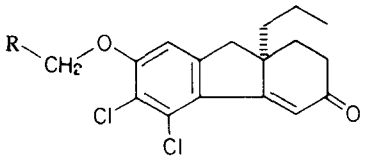
There are a number of characteristics of organic molecules alleged to beneficially affect the penetration of the blood-brain barrier.[94–96] That minor structural changes in the structure of B-3(+) can positively influence parameters which are considered important in increasing the propensity of a drug to cross the blood brain barrier with retention of intrinsic *in vitro* activity is seen in Table XVI where compounds N and B-30(+) are compared with B-3(+). The data reveal that a decrease in protein binding by as much as an order of magnitude can be accomplished and lipophilicity can be significantly increased. The ionic character can be either anionic or cationic and the unionized component of the acid or base in equilibrium with its ion can be altered (by altering the pKa). Finally, the penetration rate of a lipoidal tissue like rabbit cornea[97] can be varied by an order of magnitude. Other evidence of improved penetration of the blood brain barrier by compound N is the tissue distribution studies of the ^{14}C-labelled compound. A 1 mg/kg intravenous dose of ^{14}C-labelled compound N administered to cats produced appreciable levels of drug in various parts of the brain (in the range of 0.06 mg equivalents/g), whereas a 5 mg/kg intravenous dose of B-3(+) did not produce detectable levels of the drug.[89]

VII. SUMMARY

Traumatic brain injury has been identified as a medical problem of major importance for which no specific therapy is presently available. The sequence of events that follow traumatic insult to the brain have been examined in terms of the general pathology, the major anatomical site, the nature of the response, and the biochemical consequences of the phenomenon. The early step in the cascade of events subsequent to trauma involves swelling of the astrocytes and appears not only to be a critical event in the traumatic brain injury syndrome but the logical site at which to focus therapeutic intervention.

The knowledge that astroglial edema was a chloride mediated process provided the rationale for exploring the effects of chloride transport inhibitors. The development of a relevant *in vitro* assay involving the measurement of

Table XVI.
Comparison of the Chemical, Physical, Distribution and Biological Properties of B-3(+), N and B-30(+)



G.

No.	R	In Vitro I_{50} (M)[42–44]	Rabbit Corneal Perfusion Rate[97] pM/min/cm^2	pKa[a]	% Distribution in Octanol vs. 7.4 Buffer[a]	% Unbound by 4×10^{-4} M Bovine Serum Albumin[90,91]
B-3(+)	HO—C(=O)—	2×10^{-11}	7.81	3.66 ($-H^+$)	93	1.3
N	HCl · H_2N—C(=NH)—	2×10^{-12}	70.8	9.23 ($+H^+$)	98	26
B-30(+)	HOC—CH_2N(H)—C(=O)—	5×10^{-12}	—	4.35 ($-H^+$)	88	6

[a]See Table IV.

inhibitors of the HCO_3^--induced swelling of cat cerebrocortical slices provided an effective primary screening assay. The discovery of an animal model consisting of acceleration-deceleration trauma followed by a period of hypoxia provided an *in vivo* assay that bore a reasonable resemblance to human traumatic head injury. Ethacrynic acid was selected as a model compound possessing chloride transport inhibitor activity and modest *in vitro* inhibitory activity to evaluate the biological test systems. Although not statistically significant, the ethacrynic acid produced a sizable reduction in mortality in the *in vivo* assay. Furthermore, this drug produced a statistically significant increase in neurological recovery. A limited clinical study with ethacrynic acid in severely head injured patients demonstrated a significantly improved outcome (mortality and morbidity) in patients receiving the drug as compared to untreated controls.

Among the various leads that were identified, two were chosen to be described here. These compounds, [(6,7-dichloro-2-cyclopentyl-2,3-dihydro-2-methyl-1-oxo-1H-iden-5-yl)oxy]acetic acid and [(5,6-dichloro-9a-ethyl-2,3,9,9a-tetrahydro-3-oxo-1H-fluoren-7-yl)oxy]acetic acid, were thoroughly studied and their structures were systematically modified. The structural features, including the chiral characteristics of each series that markedly increased intrinsic activity as measured by the *in vitro* cerebrocortical slice assay, were identified. Some compounds with the greatest *in vitro* activity were evaluated in the *in vivo* acceleration-deceleration assay. Examples of those compounds exhibiting a decrease in mortality were A-10, A-10(+), and B-3(+) (see Tables VII and XIII). The latter compound was shown to exhibit a dose-related therapeutic effect as measured by the decrease in mortality in the cat acceleration-deceleration head injury model. The pharmacological effects of the compound were accompanied by the preservation of anatomical integrity as seen by electron microscopy. Structural changes in the molecules that produced a decrease in saliuretic activity and an increase in inhibitory activity in the cat cerebrocortical slice assay were paralleled by a corresponding decrease in $Na^+ + K^+ + 2Cl^-$-cotransport inhibitory potency and an increase in Cl^-/HCO_3^- anion exchange inhibitory activity.

As an update to this review, it should be mentioned that B-3 (+) has exhibited dramatically positive results in other species and models of brain injury as predicted.

VIII. ION TRANSPORT INHIBITORS AS A TARGET FOR FUTURE RESEARCH

This review highlights only one of a number of important medical disorders that involve aberrant water and electrolyte transport.[98] For many of these disorders, no specific therapeutic agent is available. The basic concepts that have led to the design of salidiuretic agents, as well as those exploited in our early stages of designing brain injury drugs, can serve as patterns for fashioning drugs for new therapeutic goals. Many of these drugs may function similarly biochemically but with different organ and tissue targets. The investigation of agents that modulate electrolyte transport should not only help identify the biochemical lesions that (at least partially) characterize the disorders but suggest new kinds of therapy.

A host of important ion transport systems and channels have been identified, including the Na^+-channel, the Na^+/H^+ antiporter, the Na^+/Ca^{+2} exchange system, the Ca^{+2}-channel, the Cl^-/HCO_3^- and $Cl^-/NaCO_3^-$ exchange systems, and the Cl^--channel, just to mention a few.

Cystic fibrosis is a disease characterized by abnormal electrolyte transport in various tissues, especially in the respiratory system. Electrolyte transport modifying agents appear to normalize transmembrane potential and, at appropriate doses, normalize some aspects of the disease process in patients with cystic fibrosis. An approach to cancer chemotherapy involves a theory that high intracellular sodium ion concentrations and the resulting depolarized transmembrane potential characterize the rapidly proliferating cells of many tumors.[98] A potential new class of anticancer drugs has been conceived as those that interfere with the regulation of intracellular pH, particularly by blocking Na^+/H^+ exchange.[99] Agents that selectively inhibit elevated sodium ion concentration have been shown to suppress the growth of several transplanted tumors in animals. Also, ion flux changes induced by certain diuretics have been reported to promote differentiation in certain transformed cells, including those from erythroleukemic cell lines. Electrolyte transport has been shown to play an important role in problems involving the gut, e.g., diarrhea, which can be normalized by certain diuretics and even their non-diuretic analogs. Basic studies on various blood cells have demonstrated the importance of ion transport, particularly of the proton which regulates intracellular and intracompartmental pH and, thus, controls enzyme activation. Muscular dystrophy is a disease involving increased cellular permeability to chloride, rendering a key chloride requiring enzyme, dipeptidyl-aminopepidase-I, inexcitable. Intervention with chloride transport blockers is suggested as an approach both to the understanding and to the therapy of this disorder.[98]

Basic research on the role and control of transmembrane transport of electrolytes is in its infancy. As the understanding of this phenomenon grows, so will our perception of its importance in health and disease. The blessing that it will afford the medicinal chemist will include the new avenues of therapy and drug design that it will unlock and the new challenges that it will afford.

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