Admin of DFP & Man.

Effect of CNS -

Brobeck et al

Bull J. H. Hop

1) Daily Adm of DFP developed: excessive dreaming insomnia, jittery + restless, nervousness, emotional lability, tremulousness, nightmare, headache -- mental confusion, tremor

symp not affected by neostigmine.

" diminished somewhat by atropine + benztropine

2) Normal subjects:

1-2 mg daily - IM

EEG: greater variation in potential

rate frequencies

More irregularity of rhythm

intermittent appearance of delta - 3-6 c/s,

high voltage, most pronounced

frontal lead. - rare by IV

"In general, more striking electroencephalographic changes due to DFP occurred in those subjects who showed greater lability of pattern, though within the limits of normal, as then control records."

EEG appeared after 2-7 days administration + usually followed onset of CNS symptoms. After DFP stopped, symp disappeared 1-4 days while EEG persisted 8-42 days

(760)

The onset or severity of CNS symp + EEG changes due to DFP bore no relation to the ChE activity of plasma

Early after administration (upto 3 days) - Symp + EEG changes coned be correlated with RBC ChE & 70-60%

When DFP over a long time, no correlation between CNS + RBC ChE activity

Atropine or DFP changes:

The EEG changes due to DFP inhibited in all of subjects by atropine

Atropine in normal subjects:

reduced elec activity in 1/3

dec in voltage, frequency

" in irreg in rhythm

delta in HV

Mode of action of DFP:

1) the EEG below, etc result from irreversible inhibition of ChE by DFP

2) persistence of EEG changes reflects slow regeneration of ChE in CNS

3) CNS effects of DFP — no relation to ChE activity of plasma
could be correlated to ChE of RBC only during first 3 days of DFP, suggesting that ChE enzymes of CNS and RBC may have similar sensitivity to DFP

4) Conclude that ACH does play a positive, though undefined role in CNS.

Convulsant Shock Rx by IV Acetylcholine

Harris, Parella

Arch NP 50 : 1943

IV - 70 mg Ach in water inc dose 20 mg untel convulsion produced.

Obsn: Cough → pale → pt sat up c feeling of choking
→ feel back incs.
Tonic extensor spasm + mild twitching of face
inc salivation, incontinence
Cardiac arrest. 30-50 secs.

EEG: after cardiac arrest, slowing to 2-3 cps noted in EEG.
if no cardiac arrest, no EEG change.

Post R: no amnesia
no EEG change
no Babinski
| diff from EST.

Use of Cholinergic Blocking Agent in
Cranio Cerebral Injury.

Ruge.
J Nunnery.

dogs - IV nembutal
dissected leech muscle

Confirm appearance of Ach after trauma
with high Ach. muscarinic BP -
i.e. Cardiac incr + ↓ BP

"Cholinergic blocking agents, particularly atropine
sulphate, have a beneficial effect upon
the parasympathetic responses that accompany cerebral
trauma because they block the muscarinic responses of
Ach."

Acth v Serotonin in CSF

Sachs -
J Neurol '57

1) clin test technic

In 28 cases of head trauma, 16 had Acth in signif amounts.

Massive atropine (2-6 mg) - destruct reduces Acth demonstrated

2) of 28 cases - 6 had serotonin
4 had Acth as well

Confirm Bonnet
+ serotonin.

Physiol. Studies of DCT in Schiz

Randall + Jellinek
1939

det'd Chol E. level in serum by gasometric analysis.

2 r Schiz.

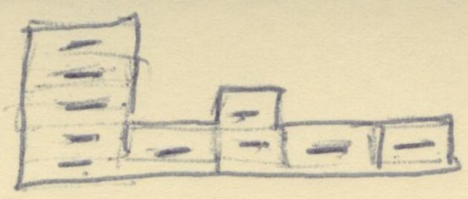
1) Schiz slightly lower serum Pre DCT than normal

2) DCT increased Post DCT, "unspecific" chemical response

3) works after R, Chol E due to emotional level.

---

for rev. of Chol E in blood
to 1937 Ginsberg et al -
Am J Dig Dis + Nut 4, 154, 1937



Cholinesterase — Augustinsson 1948

1) Blood chol E varies over time in individual

Serum chol E dec ↓ age — Heim 1940
Klin Wochschr 23, p63

Normal physiol fluct. known as reflections in Ch E

4) CHC highest in adults - lowest in children. — Berthäuser, 1940
Helv. Chim Acta 23 1071

Close relationship between relative ∫ Acet + Chol E.

3) Ch E formed in CSF — Plattner + Heuetuer 1930 Pflügers Arch ges Physiol 225 : 19

Altenburger 1937 Klin Wchr 16 : 398

Bender 1939 Amer J Physiol 126 180

Reiss + Hemphill 1948 Nature 161 : 18

Stedman + Sklan 1935a Biochem. J. 29 2107, 2563

Vahlquist 1935 Scand Arch Physiol 72 : 133

4) Chol E ∝ during development - ↓ functional develop — Nachmansohn 1938 b. J. Physiol 93 : 2

5) for Chol E in myasthenia — of Hoagland 1946 Adv. Enzymol 6 : 193

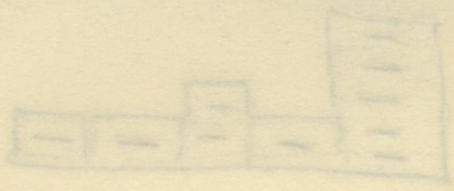
6) Low value for serum Chol E ((Cat. depr Schizophrenia) — Jones + Tod 1937 J ment Sci 83 : 702

Tod + Jones " Quart J. Exp Physiol 29 : 63

Anxiety states → high — Jones + Stadel 1939 Quart J Med 6 : 1

Ruchte + Lee 1942 J ment Sci 88 : 428, 435

7) After Insulin, lowered ChE recovers ↓ — Brett 1942. J Nat Can Med 27 : 649



Randall o Jellinek 1939 Endocrin 25 278
J hist Chem Med
Randall 1940 25 1025

Effects of DFP in Schiz + MDD

Rosenbleeth et al J Nerv. Ment Dis. 1950

cholinesterase ∴ DFP = ACh ↑

Grob (1947): EEG changes + mental symptoms.

(A)
17 schiz + 9 MDD 1 mg DFP first day; then 2 mg/day x 6 days = 13 mg.

in 4 pts - up to 43 mg.

10 normals: 6 could not tolerate more than 7 mg.

in 4 pts - atropine keeped but doses were 13, 13, 11, 7

(B)
Blood cholinesterase True: acetyl β methyl choL. (RBC)
Pseudo: benzoylchol. (Plasma)

NB: True - not stat diff. in 3 groups

Plasma: "one subject (normal) had a high level which was confirmed by repeated examinations; this subject did not exhibit any abnormal clinical signs + symptoms. His EEG, however, showed abnormalities similar to those commonly found in epilepsy."

(p48)

DFP inhibited ChoE activity by 75% in True; and 95% of pseudo in blood.

(49) "It is important to emphasize that these changes were comparable in both psychotic groups, since in some respects the clinical effects of the drug were different in the two groups."

Clin effects DFP: anorexia + nausea → vomiting

Sometimes - factor

hypotension in MD ; hypertension in schiz

Remarkable tolerance to the drug in chronic schiz

EEG

"early" when ad lib.: { lowering of amplitude, dimunition of alpha
intermittent low volt. 4-7 cps.
increased instability to HV.

after ad lib.: { increased amplitude ē inc. d.
" slow both in volt. + freq. slower (2-7 cps)
HV instability increased.
d freq. dec by 2 cps
All changes disappeared in 7 days.
EEG changes more marked in MD than schiz

in normals, EEG effects the same:

atropine: N sleep. → low amp.
theta freq. slow act. - even ē eyes open
d inc by 1-1½ cps
no change to HV.

Physiochem Changes in Brain after ECT.

Spiegel ANA 1942.

Cats in vivo. Wheatstone Bridge

1) decrease in impedance after faradic stim.

2) Inc. in conductivity

3) Leakage of ions into surrounding fluid.

CSF studies - ANA 1944 Spiegel + Spiegel-Ad.

1) Electrolytes rec in CSF after convulsion.

K^+	21½%	within 1 hr.	11.2% +3 days
PO_4^+	18½%		1.3%

but.

2) rec in conductivity.

Permeability in Brain JNMD 1941 Spiegel

S.P.gp

reduced pressure -

Killed - +5-10 mins - needle electrodes - Wheatstone Bridge

(1) → inc. permeability of surface film.

∝ to duration of convulsion.

Conc:

inc permeab. → ion exchange

cell damage

CSF ratio of $\frac{\text{non-electrolyte}}{\text{Electrolyte}}$ increase.

Action of ACH on Motor Cortex Arch NP Forster 1945

Cats . correlation of motor seizure + ACH disch.

Dial

ACH - intracisternal 10-25 mg after atropine

In these conc. - "depression of elec activity followed by long continued HV, spiking disch + tonic-clonic conv. recorded through intact skull but also exp. motor cortex

→ focal convulsion

Concludes: ACH is convulsant.

activity is independent of ~~st~~ systemic action.

≥

ACH discharges are seizure discharges.

Concludes that ACH may play important, if not the essential, role in physiologic processes of EPI.

Variation in ACH of Brain & Physiol

Rechtschaffen & Cleveland 1949

Prob ACH level in brain depends on { synthesis rate
liberation "from complex
breakdown"

If nervous activity related to
ACH, then level ↑ in sleep + Anesth
+ ↓ in convulsion

Albino rats - anesth; sleep; aware, convuls; elec
Stim of brain & conv.
↘ 35-50 v AC @ 50 cycles/sec 0.35 @ m² elec

Results - Rat brain: (method A) (method B)

Anesth -	2.5-2.7 mg/mg.	1.7-1.8
Sleep	2.4	1.43-1.47
aware	1.5-2.0	1.22-1.27
convuls	1.5-1.9	0.82-0.99
conv.	1.4-1.5	0.57
(elec Stim)		0.55

∴ ACH content of brain depends - physiol state
Anesthesia level is 300 % higher than post seizure

Treatment —
Resynthesis rate ↑ 7 mg/gm/minute

AcH in Cortex of Various Mammalian Animals.

Towers + Elliott

Studying various animals - cortex - excised. — total AcH
ChoE act.
Rate of prod of free + bound AcH

1) no obv. differences with different cortical areas in same spec.

2) Total AcH ↓ ē time after excision

Rate of ↓ same for all species

↓ Prevented by essence ; by high O_2

3) AcH act // density of cells in cortex

Effect of Anesth + Conv. on ACH Content of Brain

Am J Physiol 1950 Elliott, Swank-Hendo

Pres. theory: ACH exists in nervous tissue as inactive "bound" substance.
+ is released as "free" ACH during activity.

ACH (Bound) $\xrightarrow{\text{Activity}}$ Free ACH $\xrightarrow{\text{Chol E}}$ destroyed.

↳ Chol-Acetylase. Glucose + PO4-

Using Rats & cats: ∴ Sedation ↗ ACH content

Activity ↙ ACH

After metrazole or fibrillation, free ACH always up in CSF (to 3 γ/100 ml)
(∴ ACH is liberated during periods of alternating electrographic
pattern in sufficient amount to appear in CSF)

Acetylcholine and Convulsive Activity

J. Hyde, S. Beckett & E Geelhoed J N Phys 12, 1908

Cats:

By topical application of various drugs + eserine and prostigmine, and by mecholyl → convulsive activity is induced + enhanced.

Implication that ACH is important in convulsive Act.

(Eserine or prostigmine applied IV or hypody in doses too small for BP or HR exerted facilitatory effect.

DFP similarly applied had no effect.

Acetylcholine and Neuronal Activity

I Craniocerebral Trauma

Towers + McEachern

1) 11 patients

1) Only pts. ACH in Trauma } 0.2 → 100 mg%
ECT
+ EPI

2) Cholinesterase showed reversal of normal
track pattern in Trauma + ECT
i.e. Spec. Chol. E ↓ + decrease in
nonspec "E ↑ Total Chol E
Activity

3) The ACH level and
Extent of CholE reversal } of such Severity of cerebral dam
" " EEG

Const. ECT

			CholE ↓ MCH/ACH	CholE ↓ BCH/ACH
(a)	Pre EST-	0 ACH	= 34-36	= 9-18
(b)	5 EST	0.2 "	29	30
	6	1.0	18	32
	6	0	28	30
	7	0	35	18
	3	0	33	29
	3	0	36	30

It is interesting that this pt was the only one of the ser to show no response to Ach

II Epilepsy   Tower & McEachern

119 samples 109 Pts.
56 EPI   53 No EPI

1) ACH present in 8 non-EPI (15%)
of these 6 were Trauma cases

2) 77% (44) of EPI has pos CSF ACH
0.02 → 5.0 μgm %   av - 1.0 μgm %

3) ACH related to freq. of seizures –
occurrence –
extent of EEG abn

| 100% ? Pt who had daily seizures had pos ACH
| Samples of CSF ictal and post-ictal 100%, inter ictal - 70% } pos.

no relation to cholinesterase value
medication
type of EPI

Conclude: Since ACH associated c̄ seizures & not c̄ changes in cholinesterase, believe ACH not due to seizure itself but related to basic process

? ACH causative or reflection of other events

of Welsh on method
vol. Acht

Acht and Neuronal Activity in EPI — Cone, Tower + Lee Earl.

Ref: Pope et al ARNMD 1946 Proc. 26 : 1947

CSF shades ump polarized ventricle of mollusc Venus mercenaria

Non EPI -	17% Act Pos.	83% neg.	N=64
EPI	77% "	23% -	N=57

In discussion Dr. Tower emphasizes:

1) EST in Trauma more than EPI

2) In trauma changes in Chalinesterase -

in EPI - none

Presence and Action of Acetylcholine in Experimental Brain Trauma

Bornstein, Murray B.

J. Neurophysiol. 9: 347–366, 1946 (Sep)

In exper. head trauma, Ach found in CSF in quant. 2.7–9.0 Gamma % - may persist as long as ~~=~~ 48 hrs. Termed "free Ach"

EEG: 1) Intense neuronal discharge
2) Transient flattening of evoked electr act
3) Prolonged period of abnormality – paroxysmal high amp. sharp waves of 6.7/sec to 16.20 c/s

Behavior: tonic clonic seizures
apnea ; stupor
loss of ocular & corneal reflexes
loss of hopping & placing reactions.

EEG & Behav. effects abolished by atropine sulphate.

Ach does expand cortex. ① high amp sharp waves in small physiol concent. (1 gamma % or less)
② Sustained trend of Ach 7 2 gamma %.

Effect of certain Choline Derivatives on Electrical Activity of Cortex

Brunner - Hunt
and NP - 1942

Cats

under Dial

Ach, Methacholyl, Doryl → carbamyl choline chloride

→ acetyl beta methylcholine

Ach: 2½-10% applied topically → inc. voltage change in freq - 0 to 4 cps
↗ 30 cps

marked fluctuation

Response localized

After removal of drug, response persisted for few minutes varying with dosage
+ reversible
neutral effect

(a) "muscarinic" effect: IV atropine (1 mg/kg) had no effect on the EEG effect of Ach

(b) Ach potentiation enhanced markedly by small amounts of prostigmine

MeCh: identical response to Ach 1.25-5% sol'n.

CaCl: " " " Ach 0.3% " — to 0.08% sol'n.

↘ unaffected by cholinesterase.

Other EEG effects noted, convulsion not produced because of dual
- It is the muscarinic effect of Ach that is related to seizure, not the
muscarinic effect. (vasodilator)

N.B. CO_2 → cerebral vasodilator → no EEG effect
in fact, it raises cerebral threshold evoked

Effect of Eserine, acetylcholine & atropine on the EEG
Miller, Stavraky, etc. Am J Physiol 1938

Abst of Proc. Amer Physiol Soc.

Cats under dial; rabbits - dial or local

1% Eserine : topical : inc frequency - many small & medium sized waves appear - amplit of lg waves diminishes.
assoc. musc. responses in contralateral limbs
Opposite hemisphere EEG - ok.

0.2-1% ~~Eserine~~ ACh to eserinized cortex in 2-3 mins - rapid waves of wide amplitude.
Rabbit under local → mastication, trembling of vibrissae, & general body twitching

under dial - EEG same but motor effects are less

For EEG effect: Eserine + few minute & ACh
ACh alone → n.g. (or) under local → many small waves
ACh + Eserine → n.g.

0.1% Atropine to eserinized cortex - periods of intense hyperactivity separated by intervals of smaller amp. waves.
hyperactive waves coincide with periodic contractions of opposite limbs.

Effect of eserine + ACh is attributed to enhanced } synaptic transmiss.
atropine " " " diminished }
Interprets results that ACh is chem transmitter at synapse.

Effect of Atropine on Cortical Potentials

Brudereburk + Core 1951

study of atropine effects on brain stimulated by topical application of curare and penicillin, compared to brain activated by eserine

Understanding discrepancy in the theory which involves Ach in the mechanism of convulsions is the failure of atropine to have a beneficial effect in clinical convulsions.

∴ curare + penic. used to get convulsions by means other than accumulation of Ach

(A)

Atropine in normal cats under curare : 0.4 – 1.2 mg/Kg.

inc in amp. + slowing of waves. 2-4 cps. cats asleep.

∴ activation (topping) → low voltage, fast, irregular.

Eserine → 0.25 mg/Kg only low voltage fast
no activation effects

(B) Penic } → explosive spiking within x min of application to cortex
Curare }

Ach does not cause spiking unless Ig amounts (10%) are applied or brain pre-treated ∴ eserine.

Atropine added to Penic or curare spikes → typical grand mal pattern.
gradual (5-35 minutes)
But atropine has opposite effect on Ach spikes.

(c) Exercise + { Pento / Curare } spikes → inhibited frequency + amplitude of spiking.

or adds a slow component so that record resembles just real-

Conclude: Atropine may inhibit spiking by ACH but potentiates spiking due to other stimulating drugs.

∴ at least 2 mechanisms for initiating convulsions.

Include that compounds containing quaternary nitrogen do not significantly affect the brain - do not pass BBB

e.g. Curare - no effect IV - exc topical

Neostigmine

Adrenalin and Acetylcholine in

~~Action~~ ~~of~~ ~~the~~ Nervous System — Burn — Physiology

1) Adr. + Ach in CNS have different effects than elsewhere

In body, Adr + Ach are antagonistic

In the Nerv Sys - Adr has little action of its own

But it "exerts a powerful influence in modifying Ach"

"... in low conc. Adr augments Ach

" high " Adr depresses Ach

2) SP cord - in the presence of adrenalin, Ach may in SC cause motor discharge - Transmission affected by conc of Adr.

3) Neuromuscular junc - ⓐ trans. fm nerve to muscle improved by Adr or sympathetic.

ⓑ potentiates action of prostigmine

4) Symp ganglion - Adr. in low conc improved ganglionic trans.

" " high " depressed "

In perfused ganglia (Bülbring - J Physiol. 103 : 1944) transmission of submax stimuli increased by small amount adrenalin. depressed by ~~small~~ amount

The effect is due to potent. of Ach by Adr.

"If the action of acetylcholine in the central neurons of the brain, is indeed modified by adrenaline, it is conceivable that this modification is the basis of changes in nervous reaction, and even in behavior, which occur in emotional states when an abnormal concentration of adrenaline is present."

Atropine in the Treatment of Closed Head Injury

Arthur Ward Jr.

J Neurosurg 7 : 398-402, 1950

Refers to Towers & Bechern - 3 pts demonstrated
direct relation between ACH, CH-Est, EEG & clin.

Adm SC atropine 0.1 mg/Kg - 20 cases -
some improvement in many cases

Convulsions & ACH Toda 1953

1) Hypothesis that ACH accumulation is universal factor in initiating conv.
is questioned by: 1) ACH not always accumulated before conv
2) EST conv not preceded by ACH inc.
3) Atropine inhibits convulsions due to ACH
+ potentiates effect of some agents inducing conv.

→ cf Funderburk - EEG jour. 3: 213, 1951 ✓

Prot. Rats

Conv. induced by Metrazole

Precon. + conv. levels of ACH determined

Control: 3.70 μg / gm brain

Conv: ACH ↗ preconvulsive

decreased grad during convulsion

Concl: 1) conv. do not occur as long as ACH brain is below certain limits.
2) add'l factor is requisite.
→ this factor is prob excess ACH in conv. induced by physostigmine
" " " " not " " " " " EST or NH_4Cl

During conv. ACH ↓ because synthesis of ACH is inhibited by
metabolites as NH_4^+

Exper Prod of Elec Major Conv. Patterns. Freeman Bales, Wellesley, Mass.

Am J Physiol 146

Reviews studies of relation of ACH to seizures

1) Diamberta 1946 used ACH for shock

2) Meller, Stavraky & Woonton - sensitized brain ē essential by ACH → spikeup J-Neurophys. 3 : 1940 Am J Physiol 130 : 1942

3) Chatfield & Dempsey - prepared brain ē prostigmin & exposed spikes

Atropine prevented spikeup; or if present, could be eliminated by atropin

4) Brenner & Merritt (Arch NP - 1942) ACH to cortex → elec changes like EPI

DFP (Di-isopropyl fluorophosphate) - an irreversible anticholinesterase

Rabbits - IV DFP.

→ high ampl. rapid freq. waves N states epilept

Other patterns

These patterns are work ↓ Cholinesterase

"Because of the destruction of Cholinesterase it is probable that the convulsant patterns are produced by the excessive accumulation of a normal metabolic product of the brain, namely ACH, an interpretation supported by the prevention of the seizure patterns by the injection of atropine."

Torda, C. Ammonium Ion - J Pharm Exp Ther 107 - 1953

?- Inc. NH_4 in brain produces convulsions

Rats.

{ Conv. occur without rise in NH_4
{ Prolonged conv induced NH_4^+ ↑ -

∴ NH_4 ↑ absent ∫ rise central activity

1) ACH content ∫ brain ↓ during Convulsion

2) Convulsions cannot be maintained when ACH ↓ below normal

3) NH_4^+ ↑ during conv.

↓ Choline acetylase by 50% without changing Chol Estase

∴ dec in ACH

∴ NH_4 ↑ leads to term ∫ conv.

A

Central Homeostatic Mechanisms in Schizophrenia. Denny-Brown, P. Loe, J. Threlkeld, and M. Waddell, J Ment Sci 97: 111–131, 1951

Hall et al
J ment Sci

2 Tests of Homeostatic function: ① levels of BS, plt. Cor, etc are determ
② measured stress – type of response – ⓐ Restore steady state
ⓑ prompt or delayed

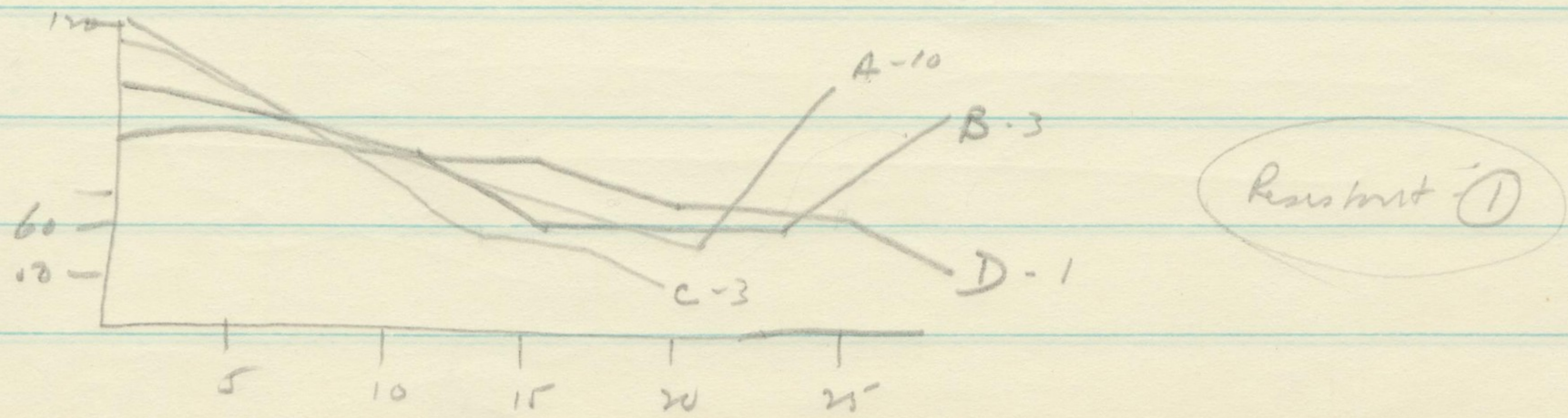
? defective homeostasis in schiz–

Hoskins: Simple tests of levels show no abnormality
the capacity to hold to steady state is defective.
i.e. variability is greater in schiz than normals.
e.g. Lack of responsivity to IV adrenalin
(tolerance) of lg doses of thyroid
Poor control of temp.
"a sluggishness of sympathetic reactivity

Stress test: Ins. Hypoglycemia–
Insulin IV. 1/2 unit/lb Bld sugar falls for 20-25
mins. Then rises sharply 3-5 mins. Re-
turns about the level.
Rise in bld sugar result of adrenalin discharge –
as bld sugar ↓, Brain waves slower — Aut NS excited pupils
Pulse & mass symp. discharge.

Conclusion: Hypoglycemia { depresses cortex –
excites autn. centers

Normal:



Resistant ①

Normal

Change: EEG slowed by 1-1½ cps

5-6 cps

Skin resist ↓ HR ↑

Schiz: Level of BS at which 5-6 cps activity appears is signif lower in schiz than in normal

Time is signif later

Relation between EEG - Bl. sug + GSR altered in sch

In all schiz the theta anticipated the adrenal discharge

Schiz show absence of response to EEG, GSR + HR

Dr Hill: "Since in 41 out of 51 of the present experiments the appearance of theta rhythm in the EEG anticipated the sympathetic adrenal discharge, this change in the electrical activity of the cortex may well be the sign of reduced cortical restraint on the activity of ~~the~~ lower autonomic centers." p130

Cholinesterases in CSF

Toever + McEachern
Can J Res - 27 135 →

1) 2 types - ACH is hydrolyzed by both
one type hydrolyzes mechalyl (= true Ch.E)
" " " Benzoylcholine (= nonspecific)

2) Concentration -

$\frac{ACH}{ACH} = 100$ Spec. Chol = $\frac{MeCh}{ACH} = 30-60$ $\frac{BCH}{ACH} = \pm 1$

Nonspec Chol = $\frac{BCH}{ACH} = 50-250$ $\frac{MeCh}{ACH} = 1-5$

CSF was obtained in PEG or LP refrigerated

Method - Warburg

Results: CSF = - Rate of hydrolysis of ACH = 0.12 - 0.89 mm³/60m/ml/hr
MeCh = 0.02 - 0.30

① Rates: Substrate/ACH for ACH = 100
MeCh = 8-52
Beng = 0-43

Benzoyl = 0.00 - 0.19

Average $\frac{MeCh}{ACH} = 33$

$\frac{BCH}{ACH} = 17$

② No significant diff of ACH or MeChE or BeChE in cells
in Rbc (if under 2000/Rbc/mm³

③ In Trauma + EST - Spec Cholin E (MeCh) ↓
Nonspec " (BCH) ↑↑

There is a definite correlation between the activity of the cholinesterase

abnormalities and the degree of trauma"

Studies on Cholinesterase - Nachmansohn & Rothenberg

1. On the Specificity of the Enzyme & in Nerve Tissue

J Biol Chem 158 : 653-666, 1945

1) Specif. Cholinesterase is found in CNS

Esterase in all nerve tissue is predom. cholin E.

2) Serum Esterase is non-specific

RBC " " is ACH specific

Effect of convulsant + anticonvulsant on ACH metab

Torda - Wolff 1947

1) convulsion reducing agents cause an accumulation or increase of ACH by increasing synthesis (pecrotoxa, pentamethylene tetrazole) or by decreasing hydrolysis (strychnine, morphine)

2) Convulsion producing agents potentiated by ACH or physostigmine

3) Other mechanisms than ACH must exist - as convulsion follow camphor, digitalis · even though ACH depressed

4) Anticonvulsants decrease ACH synthesis ex Bromide + trimethadione.

Serum choline esterase & Anxiety & Depress. — D. Richter & Lee 1942.

Measured ACH hydrolysis using Warburg tubes.

Ref. Tod & Jones 1937

12 Normal	35-112	± 22	mean = 74	vol CO_2 liberated by 1 cc serum @ 37° in 1 min
12 Anx.	77-162	± 24	" = 104	
12 Surg	46-105	± 20	" = 75	

Believed inc. Chol E due to high level in tissue — not due to general inc. in Protein.

Not affected by B₁ narcosis

Decreased during hosp. in 8/10 pts. under Rx for anxiety

Depress.	mean act.	Range	SD
N	75	~~[illegible]~~ 35-112	~~35~~ 21
Anx	95	35-137	20
Anx + Depr.	103	59-126	19
Depr	96	65-169	23
Post Conc.	108	66-126	23

Jones: Cholesterase in Blood Serum J Ment Sc 1937

142 Cases

Normal: 52–114 units (ml CO_2 / mm / ml serum)

① Inc: anxiety neurosis (7)
agit Depr. (3)
Manic (3)
exapt Sch (2)
} anxiety high. (auton. overactivity)

Dec: Epi -(5)
Mel. Stupor (3)
Depr) (2)
Post encep (1)
} Stupor no auton overact

② Cholinesterase unaffected by eserine.

③ " " " prostigmine.

④ Single dose of daryl → no effect
daily " " " x 10 d. → inc Cholinest. act.

Kaplan et al: J lab clin med.

(a) Study of Enzymes in Normal + Partial CSF

113 fluids - 77 Pts. Trypsin, antitrypsin, phosphatase, lipase, tributyrinase, esterase + amylase.

1) Normal: small amounts of beta-glycerophosphatase + amylase are. lipase, tributyrinase + antitrypsin.

2) Tbc meningi. ↑ trypsin, amylase, antitrypsin, etc.
No esterase
etc.

BTS: invaded meninges or recent → ↑ enzymes.

(b) Source of Enzymes in N. + Path CSF
1) Blood plasma + neural tissue -
?? which.

1) where cells in CSF - lg amount due to cells

Spiegel Adolf - et al Jed Proc 6 - 1947

(a)

"There experiments seem to indicate that enzymatic processes play a part in the genesis of changes of nucleic acid metabolism of the brain following electroshock"

studied nucleic acids -

optical absorption (1)

extinction coeff (E_{2650})

1) at 2650 Å - E = 2.0 ± 0.5 for schizo

" E = 1.24 ± 0.59 " normals } electric ab. = nucleic acid in CSF

Following 3-10 Conv. - (Pubescent) dec or no chg in E

" " - (Medicaft) - first depr. in E, then rise persisted for days → wks.

"These observations are taken to mean changes in nucleic acid metabolism related to the electroshock convulsions.

Spiegel-Adolf

Fed Proc 1946.

Spectrophotometry of cmc. CSF (Ref: Fed Proc 4: 105/1945)

If CSF kept standup, selective abs. band at 265 mμ dec + deaps.
Temp inc reactn

By studying nucleic acid of animal (Difco) + plant (schmidt)

mcmb. i CSF

same curve when CSF put through but not i controls.

∴ enzymes put chromatolysis in CSF

Ph. 174800

MECHOLYL® CHLORIDE

(METHACHOLINE CHLORIDE U.S.P., MERCK)

SHARP & DOHME

MECHOLYL* Chloride produces the same physiologic response as does acetylcholine, which, when released at nerve endings, produces parasympathetic stimulation. In therapeutic doses, MECHOLYL slows the heart rate, lowers blood pressure, constricts the bronchioles, dilates the peripheral blood vessels, constricts the pupils, increases intestinal tone and peristalsis, causes salivation and flushing, and stimulates the detrusor muscle of the bladder. In general its effects are the opposite of those produced by epinephrine. Its action is much more prolonged than that of acetylcholine and it is, moreover, devoid of the nicotine-like effect of that substance.

METHODS OF ADMINISTRATION

MECHOLYL Chloride is a potent substance and careful consideration should be given to its dosage and method of administration. For

*MECHOLYL is the registered trade-mark of MERCK & CO., Inc., for its brand of methacholine.

stopping an attack of paroxysmal auricular tachycardia it should be given by subcutaneous injection (*never by intravenous or intramuscular injection*), and the same method of administration may be used in treating scleroderma, chronic ulcers, Raynaud's disease and other vasospastic states, although in the latter conditions better and more prolonged effects are obtained when it is administered by the method of ion transfer (iontophoresis). For administration by mouth the less hygroscopic MECHOLYL Bromide is supplied in tablet form.

MECHOLYL Chloride is supplied in ampuls containing 25 milligrams (0.025 gram) of the powder. Solutions for subcutaneous injection are prepared by dissolving the drug in sufficient sterile distilled water to make it possible to measure accurately and administer easily the dosage desired.

WARNING

Injections of MECHOLYL Chloride should be given subcutaneously only. Injections should never be given intravenously or intramuscularly.

PRECAUTIONS

The patient should be lying down during the administration of MECHOLYL Chloride to minimize the effects of lowered blood pressure.

Atropine intravenously immediately terminates the action of MECHOLYL. A syringe containing a suitable dose of atropine sulfate [0.6 milligram (1/100 grain)] should be available for immediate intravenous injection if the dose of MECHOLYL Chloride causes undesirable symptoms.

Overdosage of MECHOLYL may produce momentary cardiac arrest. The Trendelenburg position, to give the cardiac center the benefit of any circulation present, is sometimes beneficial in such an emergency.

Since MECHOLYL constricts the bronchioles and may produce an asthmatic attack in those subject to this condition, it should be used with extreme caution, if at all, in cases where there is a history of asthma or hypersensitivity.

Substernal pain following the administration of MECHOLYL is said to be rare. However, the use of this drug in patients subject to angina pectoris is not recommended.

USE IN PAROXYSMAL AURICULAR TACHYCARDIA

One of the most efficacious uses of MECHOLYL Chloride is in terminating attacks of paroxysmal auricular tachycardia. It is, however, not effective for prophylaxis or for continued treatment in cases of frequent recurrence of the arrhythmia.

It is not recommended for the treatment of auricular fibrillation, auricular flutter, or paroxysmal ventricular tachycardia.

DOSAGE

The initial subcutaneous dose of MECHOLYL Chloride should be limited to 10 milligrams (0.01 gram) to test the patient's tolerance. Careful preliminary testing of the patient with a small dose will not nullify the effect of a subsequent dose, and is advisable if there is any doubt of the patient's ability to tolerate the drug.

In treating paroxysmal auricular tachycardia in patients under twenty years of age, 10 milligrams given subcutaneously usually terminates an attack. In older patients, 20 to 40 milligrams may be required; obese patients sometimes require more.

Slow absorption of the drug due to inadequate local circulation may interfere with the therapeutic response. If the attack is not terminated in two minutes, compression of the vagi, together with gentle massage at the site of injection to promote absorption, is suggested. Conversely, if absorption is found to be too rapid, further absorption may be retarded by applying a tourniquet above the site of injection. The effects of MECHOLYL may be terminated immediately by atropine.

A second and larger dose (if that given first fails to interrupt the attack) may be given 20 to 30 minutes later, providing no severe reaction has occurred following the first dose.

Quinidine in moderate doses (not more than 0.2 gram four times a day) usually does not impair the MECHOLYL effect. Larger doses tend to inhibit its action, although MECHOLYL has been known to "break through" the depression of quinidine.

OTHER USES OF MECHOLYL CHLORIDE

MECHOLYL Chloride has been used (by subcutaneous injection) in a number of other conditions, particularly in certain vasospastic diseases, such as Raynaud's disease, in chronic ulcers, and in scleroderma. If a test dose of 10 milligrams of MECHOLYL Chloride has been well tolerated, the subsequent dose may be increased cautiously up to 25 milligrams (0.025 gram). In these conditions, however, the much more prolonged effect produced by MECHOLYL Chloride administration by the method of iontophoresis (ion transfer) or by the oral administration of MECHOLYL Bromide Tablets is preferred.

MECHOLYL CHLORIDE is supplied as follows:

For subcutaneous injection—

Boxes of 6 ampuls each containing 25 mg. (0.025 Gm.) of the dry powder.

For oral administration or administration by the method of ion transfer (iontophoresis)—

1 Gm. bottles.

10 Gm. bottles.

MECHOLYL BROMIDE (for oral administration only) is supplied in

Boxes of 24—200 mg. (0.2 Gm.) tablets

Bottles of 500—200 mg. (0.2 Gm.) tablets

SHARP & DOHME

SHARP & DOHME

Philadelphia, U. S. A.

DIVISION OF MERCK & CO., INC.

P- IB-413 Printed in U.S.A.

'ANECTINE'®

CHLORIDE BRAND
SUCCINYLCHOLINE CHLORIDE
INJECTION

20 mg. in each cc.
Multiple-dose vials of 10 cc.
(*for intravenous use*)

CAUTION: Succinylcholine Chloride may produce respiratory depression as a result of its neuromuscular blocking action. While respiratory depression is usually of short duration following a single dose of the drug, or following cessation of continuous administration, there may on occasion be more prolonged respiratory depression requiring adequate respiratory exchange of oxygen by the administration of supplemental or controlled respiration. The drug should be used only by those skilled in the administration of supplemental or controlled respiration and facilities for this procedure and the administration of oxygen should always be immediately available. Neostigmine and other anticholinesterases, as well as edrophonium (Tensilon) do not antagonize the action of Succinylcholine but, on the contrary, prolong its effect. They are, therefore, contraindicated as antidotes for Succinylcholine. Intravenous injection of procaine likewise may prolong the action of Succinylcholine.

'ANECTINE' Chloride brand Succinylcholine Chloride Injection is an ultra-short-acting skeletal muscle relaxant; that is, following intravenous injection of small doses (10 mg. to 20 mg.) the relaxation persists approximately three minutes. The drug is thus ideally suited for endotracheal intubation and other short surgical procedures. The quick return of spontaneous respiration is a definite advantage. For more prolonged relaxation 'ANECTINE' may be given by continuous intravenous drip; tachyphylaxis does not occur and cumulative action is not ordinarily seen. The degree of relaxation may be controlled by adjusting the rate of flow of the solution. Upon stopping the intravenous drip, spontaneous respiration ordinarily resumes within a minute and recovery is complete within 5 minutes.

CHEMICAL PROPERTIES

Succinylcholine chloride, also referred to as diacetylcholine chloride, is a white, odorless, crystalline substance which is readily soluble in water. Chemically it is succinic acid bis (β-dimethyl-aminoethyl) ester dimethochloride, and its formula is as follows:

$$\begin{array}{l} \qquad\qquad\qquad\quad Cl \\ \qquad\qquad\qquad\quad | \\ CH_2COOCH_2CH_2N(CH_3)_3 \\ | \\ CH_2COOCH_2CH_2N(CH_3)_3 \\ \qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\quad Cl \end{array}$$

The ester linkage is rapidly hydrolyzed in alkaline solutions but is relatively stable in acid solutions. In order to promote stability, solutions should be kept under refrigeration. It appears that succinylcholine is rapidly hydrolyzed following its injection and that this accounts for its extremely short duration of action and the rapid recovery of normal muscle tone.

PHARMACOLOGICAL ACTION

'ANECTINE' causes muscular paralysis by producing a blockage of nervous transmission at the myoneural junction. This action was first reported by Bovet et al.[1] Independent studies at The Wellcome Research Laboratories have been conducted on the synthesis[2] and pharmacology[3-7] of the drug. de Beer and his associates[3-7] have found that doses as low as 0.05 mg./Kg. given intravenously to cats are effective in producing muscular relaxation, and that intravenous doses of 0.1 mg./Kg. or more produce prompt and complete muscular paralysis which is characterized by short duration of action and extremely rapid recovery. Repeated injections produce reproducible and predictable muscular paralysis, neither tachyphylaxis nor significant cumulative effects being seen.

When given by intravenous drip, a predetermined degree of relaxation in a cat (sciatic nerve-gastrocnemius muscle) could be closely approximated by adjusting the rate of flow of the solution.[6]

The administration of doses of 'ANECTINE' sufficient to produce complete neuromuscular blockade has not caused any significant change in blood pressure (except for the typical asphyxial pressor response in the absence of adequate respiration). No evidence of any histamine-like depressor action has been found, thus differing from observations with d-tubocurarine. The ECG of the cat was unchanged during a 2-hour infusion maintaining complete paralysis.

Acute toxicity studies in albino mice showed the intravenous $L.D._{50}$ to be 0.55 to 0.59 mg./Kg. Complete paralysis resulted, with marked dyspnea and anoxia; death was apparently due to respiratory failure. Those mice which survived the initial symptoms exhibited disappearance of anoxia and dyspnea in 2 minutes and had completely recovered within 30 minutes. Chronic toxicity studies on albino rats showed that the intraperitoneal injection of 1 mg./Kg. or less, twice daily over a period of 4 weeks, produced no evidence of toxicity.

An important difference between 'ANECTINE' and d-tubocurarine is that the former is not antagonized by anticholinesterases. On the contrary, such drugs as physostigmine, prostigmine (neostigmine) and procaine prolong the action of succinylcholine. This would support the theory that succinylcholine is hydrolyzed by cholinesterases and that interference with this enzyme action results in persistence of activity of the drug. Edrophonium (Tensilon) also prolongs the action of succinylcholine.

CLINICAL INDICATIONS AND DOSAGE

Short Duration: 'ANECTINE' Chloride brand Succinylcholine Chloride Injection is indicated for the production of muscle relaxation during surgical procedures,[8,9,13,15-17] and in conjunction with electroshock therapy.[13,14,16] In view of its very short duration of action (usually about 3 minutes following a single intravenous injection) succinylcholine is ideally suited for procedures requiring only brief relaxation, such as endotracheal intubation, endoscopic examinations, orthopedic manipulations, short surgical procedures such as tonsillectomies, and electroshock therapy. As described previously, intravenous administration of the drug produces relaxation within a minute, which lasts about 3 minutes and is quickly followed by recovery of spontaneous respiration in those cases where apnea has occurred.

Dosage for Short Procedures: The average dose for relaxation of short duration is 20 mg. (1 cc.) 'ANECTINE' Injection given intravenously (Foldes[8]). The optimum dose will vary among individuals and may vary from 10 to 30 mg. for adults (0.5 to 1.5 cc.). Following administration of doses in this range, relaxation develops in about 1 minute; maximum muscular paralysis may persist for about 2 minutes, after which recovery rapidly takes place within the next few minutes. However, very large doses may result in more prolonged apnea.[19-21]

Obviously, facilities for supplemental or controlled respiration with adequate exchange of oxygen should be available at all times. In order to avoid carbon dioxide accumulation and hypoxia, supplemental or controlled respiration should be provided during respiratory depression without waiting for the development of apnea.

Prolonged Relaxation: Although 'ANECTINE' is short-acting, prolonged relaxation may be obtained by repeated injections or, preferably, by maintaining a continuous intravenous drip.[8,30] By adjusting the rate of flow, the desired degree of relaxation may be obtained and maintained, and the degree of relaxation can be changed within 30 seconds by changing the rate of flow. Upon stopping the flow of the intravenous drip solution, relaxation quickly disappears. In those cases where respiration has been depressed it usually returns to normal within a few minutes upon stopping the intravenous drip.

Dosage for Long Procedures: The average dose for continuous intravenous infusion is 2.5 mg. per minute for adult patients. For convenience in preparing solutions for intravenous drip there are available 'Anectine' Chloride Solution, **50 mg. per cc., 10 cc. ampuls and 100 mg. per cc., 10 cc. ampuls.** The contents of one 500 mg. in 10 cc. ampul may be added to 500 cc. sterile isotonic saline solution to prepare an 0.1% (1 mg. per cc.) 'Anectine' Chloride Solution; the contents of one 1 Gm. in 10 cc. ampul may be added to 1,000 cc. to prepare an 0.1% 'Anectine' Chloride Solution. This concentration is suitable for continuous intravenous infusion. See **literature accompanying 'Anectine' Chloride Solution, 50 mg./cc., 10 cc. ampuls, and 100 mg./cc., 10 cc. ampuls** for details regarding use of this product for obtaining prolonged relaxation. Solutions for intravenous drip may also be prepared for a dilution of 'Anectine' Injection, 20 mg./cc. in appropriate proportions.

NOTE: Succinylcholine is rapidly hydrolyzed by alkaline solutions and therefore loses potency rapidly if mixed with thiopental sodium (pentothal sodium). Such mixtures, if used at all, must be used within a few minutes of preparation; however, separate injection of 'ANECTINE' is preferable. Succinylcholine chloride is quite stable when stored under refrigeration. On long standing at room temperature potency gradually decreases; however, solutions may be kept as long as 3 months at room temperature without significant loss of potency as determined by biological assay.

CONTRAINDICATIONS AND PRECAUTIONS

The drug should be used only by those skilled in the administration of supplemental or controlled respiration and facilities for this procedure, including adequate respiratory exchange with oxygen, should always be immediately available.

'ANECTINE' is not an anesthetic agent and should not be regarded as a substitute for anesthesia; its use does not take the place of giving an adequate amount of anesthetic agent.

Some anesthesiologists believe that rapid injection is responsible for the muscular twitching that is seen just prior to relaxation. These fasciculations may be due to the rate of injection of the drug, and may be minimized or avoided by giving the injection more slowly.[8,23]

While respiratory depression is usually of very short duration following a single dose of the drug, or following cessation of continuous intravenous administration, there may be an occasional case, especially with excessive doses, more prolonged respiratory depression [19-21] requiring controlled respiration and the administration of oxygen.

The duration of the effect of 'ANECTINE' may depend on plasma-cholinesterase activity.[24,26,27] Patients with severe liver disease, severe anemia, severe malnutrition, and possibly those suffering from polyphosphate insecticide poisoning may have a decreased plasma-cholinesterase activity which may intensify and prolong the action of 'ANECTINE', especially if large doses are used.[28,29] In such cases, in addition to the usual measures of controlled respiration and administration of oxygen, it may be desirable to administer plasma or whole blood for the purpose of restoring cholinesterase activity.[28]

Neostigmine and other anticholinesterases, as well as edrophonium (Tensilon), do not antagonize the action of 'ANECTINE', but on the contrary prolong its effect. They are therefore contraindicated as antidotes for 'ANECTINE'. Intravenous injections of procaine likewise may prolong and intensify the action of 'ANECTINE'.

There is evidence that intraocular pressure is increased slightly following injection of 'Anectine'.[31] This effect is seen immediately after the injection and during the fasciculatory phase; it subsides as complete paralysis supervenes; it appears to be the result of brief contraction of the extraocular muscles. This suggests that 'Anectine' should be used with caution, if at all, in intraocular surgery. The opinion is expressed that the effect is probably not sufficient to contraindicate the drug in general surgery or electroshock therapy for patients with glaucoma.

BIBLIOGRAPHY

1. Bovet, D., Bovet—Nitti, F., Guarino, S., Longo, V.G., and Marotta, M.: Pharmacodynamical property of certain derivatives of succinylcholine with curare-like action: esters of trialkylethanolamine of dicarboxylic aliphatic acids. Rendiconti Istituto Superiore di Sanita 12:106, 1949.
2. Phillips, A.P.: Synthetic curare substitutes from aliphatic dicarboxylic acid aminoethyl esters. J. Am. Chem. Soc. 71:3264, 1949.
3. Castillo, J.C., and de Beer, E.J.: Potentiation of curarizing action of diacetylcholine (succinylcholine) by aliphatic dicarboxylic acid aminoethyl amides. Federation Proceedings 9:262, 1950.
4. Castillo, J.C. and de Beer, E.J.: The neuromuscular blocking action of succinylcholine (diacetylcholine). J. Pharmacol. & Exper. Therap. 99:458, 1950.
5. de Beer, E.J., Castillo, J.C., Phillips, A.P., Fanelli, R.V., Wnuck, A.L., and Norton, S.: Synthetic drugs influencing neuromuscular activity. Ann. New York Acad. Sci. 54:362, 1951.
6. Wnuck, A.L., Norton, S., Ellis, C.H., and de Beer, E.J.: Production of controlled neuromuscular block by infusion of diacetylcholine. Federation Proceedings 11:403, 1952.
7. Ellis, C.H., Norton, S., and Morgan, W.V.: Central depression by drugs which block neuromuscular transmission. Federation Proceedings 11:42, 1952.
8. Foldes, F.F., and McNall, P.G.: Succinylcholine: A new approach to muscular relaxation in anesthesiology. New England J. Med. 247:596, 1952.
9. Brucke, H., Ginzel, K.H., Klupp, H., Pfaffenschlager, F., and Werner, G.: Muscle relaxing effects of bis-choline ester of dicarboxylic acid in narcosis. Wien. klin. Wchnschr. 63:464, 1951.
10. Ginzel, K.H., Klupp, H., and Werner, G.: Pharmacology of α, ω-bis-quaternary ammonium compounds. Comparative tests with some aliphatic dicarboxylic acid esters. Arch. int. Pharmacodyn. and Therapy. 87:79, 1951.
11. Ginzel, K.H., Klupp, H., and Werner, G.: A dicholine ester with greater curare effect. Experentia 7:72, 1951.
12. Arnold, O.H., Bock-Greissau, W., and Ginzel, K.H.: Wien. med. Wchnschr. 101:492, 1951.
13. Thesleff, S.: Pharmacological and clinical tests with LT 1. (0.0—succinylcholine iodide). Nordisk Med. 46:1045, 1951.
14. Holmberg, G., and Thesleff, S.: Succinylcholine iodide as a muscle relaxant in electro-shock treatment. Nordisk Med. 46:1567, 1951. Abst. in J.A.M.A. 148:1064, 1952.
15. Dardel, O.V., and Thesleff, S.: Clinical results with succinylcholine iodide, a new muscle relaxant. Nordisk Med. 46:1308, 1951.
16. Thesleff, S., and Dardel, O.V.: Clinical report on succinylcholine iodide. Presented at 26th International Congress of Anaesthetists, London, September 3—7, 1951. Abstracted in J. Am. M. Wom. Assn. 7:58, 1952.

17. Mayrhofer, O., and Hassfurter, M.: Surgical risks in patients with cardiac and vascular disorder. Wien. klin. Wchnschr. 63:885, 1951.
18. Holzer, H.: Wien. med. Wchnschr. 102:112, 1952.
19. Harper, J.K.: Prolonged respiratory paralysis after succinylcholine. Correspondence. Brit. M.J. 1:866, 1952.
20. Love, S.H.S.: Prolonged apnea following scoline. Correspondence. Anesthesia (London) 7:113, 1952.
21. Gould, R.B.: Succinylcholine. Correspondence. Brit. M.J. 1:440, 1952.
22. Bovet, D., Bovet—Nitti, F., Guarino, S., Longo, V.G., and Fusco, R.: Investigations on synthetic curarizing drugs. III. Succinylcholine and its aliphatic derivatives. Arch. int. Pharmacodyn. and Therapy 88:1, 1951.
23. Poulsen, H. and Hougs, W.: Letters to the Editor, Lancet 2:199, 1952.
24. Foldes, F.F.: Letters to the Editor, Lancet 2:245, 1952.
25. Kay, H.T.: Letters to the Editor, Lancet 2:200, 1952.
26. Evans, F.T., Gray, P.W.S., Lehmann, H., and Silk, E.: Sensitivity to Succinylcholine in Relation to Serum-cholinesterase, Lancet 1:1229, 1952.
27. Bourne, J.G., Collier, H.O.J., and Somers, G.F.: Succinylcholine (Succinoylcholine)—Muscle Relaxant of Short Action, Lancet 1:1225, 1952.
28. Lehmann, H.: Letters to the Editor, Lancet 2:199, 1952.
29. Hampton, L.J.: Personal communication.
30. Little, D.M., Jr., Hampton, L.J., and Grosskreutz, D.C.: Diacetylcholine (Succinylcholine): A Controllable Muscle Relaxant. Presented before the Twenty-seventh Annual Congress of Anesthetists, Virginia Beach, Virginia, September 22-25, 1952.
31. Lincoff, H.A., Ellis, C.H., DeVoe, A.G., de Beer, E.J., Impastato, D.J., Berg, S., Orkin, L., and Magda, H.: The Effect of Succinylcholine on Intraocular Pressure. Am. J. Opth. 40:501, 1955.

Poloni, A.: L'Acetilcolina nel liquor dei malati di mente. Mancanze di effetto acetilcolina e azione curarosimile del liquor di schizophrenici sul muscolo dorsale della sanguisuga, Il Cervello 27: 81-104, 1951.

Translation of summary:

The author, using the method of Tower for the conservation of acetylcholine in spinal fluid, and the dorsal muscle of the leech for the test, has made the following observations in several trials, making use of the spinal fluid of 10 normal subjects and 110 mental patients, of whom 50 were schizophrenics, 10 progressive paralytics and 50 subjects with other forms of mental disease:

(1) That the spinal fluid of normal subjects contains acetylcholine in a concentration varying from $1:10^{-7}$ to $1:10^{-10}$.

(2) That the spinal fluid of schizophrenics in 94% of the cases does not contain acetylcholine but a substance which produces an action antagonistic to acetylcholine, comparable to that of "curare", in a concentration of $1:10^{-5}$ to $1:10^{-6}$.

(3) In the spinal fluid of the progressive paralytics one encounters the same curare-like substance but in a lower concentration than in that of schizophrenics.

(4) The spinal fluid of persons affected with other forms of mental sickness, as well as that of normal subjects, did not contain the curare-like substance in a discernible quantity, but only acetylcholine, which was found in greater concentration in the hystericals and epileptics, in lower concentration in senile psychotics and alcoholics.

This emphasizes the pathologic vale of the report obtained from the spinal fluid of schizophrenics and progressive paralytics and suggests the hypothesis that the curare-like substance is trimethylamine, product of the excessive catabolism of choline, of which the author has found an abnormal urinary excretion in schizophrenics.

(In the same paper, in a footnote, the author eliminates trimethylamine, since it does not have curare-like properties.)

CHOLINERGIC ASPECTS OF CONVULSIVE THERAPY

Max Fink, M.D.

From the Department of Psychiatry, Washington University School
of Medicine and the Department of Psychiatry at the
Missouri Institute of Psychiatry, University of Missouri
School of Medicine, 5400 Arsenal St., St. Louis, Missouri 63139.

Aided, in part, by USPHS grants MH-927, MH-2715, MH-07249 and
MH-11380; and the Psychiatric Research Foundation of Missouri.

IX: 2-25-66 Revised for the *Journal of Nervous and Mental Disease.*

65-8

CHOLINERGIC ASPECTS OF CONVULSIVE THERAPY

While the mode of action of convulsive therapies remains enigmatic, one theory holds that the early development and persistence of changes in brain function are requisite to changes in behavior.[18,21,22] A useful index of neurophysiological change is the appearance of high voltage electroencephalographic slow wave activity.[22,23] While the biochemistry of this activity is poorly understood, demonstrations that it is inhibited by anti-cholinergic compounds[19,20,34,66] suggest that cholinergic systems may play an active part.

The EEG patterns and the response to anticholinergic drugs in convulsive therapy are similar to experimental and clinical head trauma and to a lesser extent, to spontaneous seizures. Changes in concentration of cholinesterases in brain and spinal fluid also show many similarities in these conditions. This review discusses these observations to provide a hypothesis for the role of cholinergic changes in convulsive therapy.

The activity of acetylcholine in the transmission of nervous impulses has been extensively studied since the early descriptions by Dale[12] and Loewi.[38] A constituent of nervous tissue in a bound form, acetylcholine is liberated during the excitation process. It is rapidly hydrolyzed through the mediation of acetylcholinesterase and is rapidly reconstituted by the

choline-acetylase system.[45] Free acetylcholine has not been measurable in normal cerebrospinal fluid despite the rapid breakdown of bound acetylcholine during periods of activity and excitement.[63] But the normal cerebrospinal fluid does have measurable cholinesterase activity.[41]

Cholinergic Aspects of Craniocerebral Trauma. Free acetylcholine was found in the cerebrospinal fluid of cats within a few minutes after experimental head trauma and persisted for varying periods up to 48 hours. The quantity of free acetylcholine varied between 2.7 and 9.0 gamma/100cc and the amount was related to the degree of induced trauma.[6]

Concurrent electroencephalograms first demonstrated high voltage fast activity, interpreted as evidence of an intense neuronal discharge, which was succeeded by a short period of flattening of all recorded electrical activity. These phases were followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

The behavioral changes related to the degree of induced trauma and to the amount of measured free acetylcholine. With higher levels of acetylcholine, Bornstein reported greater degrees of EEG abnormality and greater changes in consciousness. Spontaneous post-traumatic seizures were also related to the amount of free acetylcholine measured in the cerebrospinal fluid.

Bornstein applied acetylcholine to exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma/100cc or less, high amplitude sharp waves of low frequency appeared in the electroencephalogram. When the concentration was increased to 2 gamma/100cc, the electroencephalogram flattened in a fashion parallel to the post-traumatic records.

Investigations in neurological patients by Tower and McEachern demonstrated free acetylcholine in the cerebrospinal fluid only in patients with recent head trauma, recent grand-mal seizures or after electroconvulsive therapy.[63] Free acetylcholine varied from 0.2 to 100 gamma/100cc. In assaying spinal fluid cholinesterase activity, they noted a sharp rise in the butyrylcholinesterase fraction and a fall in the acetylcholinesterase fraction in patients with head trauma and following convulsive therapy. After spontaneous seizures, however, the cerebrospinal fluid did not exhibit such inversion although it contained free acetylcholine. They concluded that the level of free acetylcholine varied directly with the degree of cerebral damage and that reversal of cholinesterase fractions was a more sensitive indicator of cerebral damage. Electroencephalograms taken at varying intervals following trauma also indicated a relation between the degree of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid.

Increased acetylcholine in rat brain after traumatic shock was also reported by Kovach, *et al.*[36] This acetylcholine activity was inhibited by the administration of atropine *in vitro*.

The electrographic, behavioral and neurologic signs of head trauma were blocked by the parenteral administration of 0.5-1.0 mg/kg atropine, as were similar clinical changes occurring after the intracisternal addition of acetylcholine.[6] Ward applied these observations to the treatment of closed head injuries. In 20 patients with varying degrees of trauma, he administered atropine subcutaneously in doses of 0.1 mg/kg, noting clinical improvement in some and a reversal of the electrographic effects in others.[67] The same changes in the post-traumatic electroencephalogram were reported by Jenkner and Lechner in a study of diethazine, another anticholinergic drug. A single intravenous dose in forty patients resulted in normalizing the abnormal electroencephalogram in twenty-two and marked improvement in six others.[33]

Similarly, in experiments of post-traumatic shock and cerebral edema in animals, Denisenko reported a blocking of the clinical changes by such anticholinergic compounds as methylbenactyzine and adiphenine (Trasentin).[13]

Thus, the amount of free acetylcholine may increase in the spinal fluid following crainocerebral trauma and the amount of

free acetylcholine, the degree and type of electroencephalographic abnormality, and changes in clinical behavior appear as interrelated phenomena, which may be reduced by the administration of anticholinergic drugs.

Brain acetylcholine and anticholinergic drugs. The effects of the direct application of acetylcholine to the central nervous system may also be blocked by anticholinergic drugs. The administration of the cholinesterase inhibitor di-isopropyl fluorophysphate (DFP) elicited high amplitude rapid frequency EEG patterns similar to status epilepticus and some post-traumatic states.[24,31,32,68] These EEG effects were blocked by small doses of parenteral atropine and scopolamine. The great increase in acetylcholine after tetraethyl pyrophosphate (TEPP) was measured and related to the toxic effects and the induced convulsions.[29,59]

Chatfield and Dempsey prepared exposed animal cortex with prostigmine and evoked electroencephalographic spike activity. The prior administration of atropine blocked the appearance of spiking, or if present, this electrical activity could be eliminated by atropine.[9]

In contrast to these findings, Brenner and Merritt applied topical acetylcholine in concentrations of 2-1/2 to 10% to the exposed cortex of cats and noted no effect on the electroencephalographic changes after intravenous atropine (1 mg/kg).[7]

The concentrations of acetylcholine in these experiments, however, were higher than the topical applications (1-4 gamma/100cc) and the intracisternal (0.2-10 gamma/100cc) injections of Bornstein.[6] Brenner and Merritt also noted electroencephalographic effects similar to acetylcholine after methacholine (Mecholyl) and carbamylcholine (Doryl) in concentrations much lower than the acetylcholine concentrations. They ascribed the increased effectiveness of these cholinergic drugs to their lack of sensitivity to cerebral cholinesterases.

These data are conflicting and further study is necessary to qualify this issue.

Cerebrospinal Fluid Acetylcholine and Seizures. One view of acetylcholine metabolism finds it in nervous tissues in an inactive and bound form. During periods of activity, acetylcholine is liberated at the cell membrane where it is rapidly deactivated by cholinesterases. The amount of bound acetylcholine is the resultant of the continuous processes of synthesis, liberation and breakdown.[15] It has been postulated that the level rises during sleep and falls during waking activity.[16,29,45,60]

Tobias *et al.* reported increased free and total acetylcholine after chloroform and pentobarbital anesthesia in rat and frog brain but no changes after strychnine or picrotoxin convulsions.[60]

Richter and Crossland measured the level of acetylcholine (micro-gamma per mg. brain tissue) during anesthesia and sleep in rat brain to be 300% higher than post-seizure levels. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (7 gamma/gm/minute).[45] These observations were confirmed by Elliott *et al.*[16] and Crossland and Merrick.[11] Giarman and Pepeu reported the increase in acetylcholine following various depressants to be roughly proportional to the degree of depression of the central nervous system and the reduction in motor activity.[29] Maynert and Buck, however, studying brain acetylcholine levels during sedation concluded that some sedatives were associated with elevated brain acetylcholine but that no rigorous relationships existed.[39] In part, this may be related to the earlier observations of McLennan and Elliott that acetylcholine synthesis measured in rat brain slices is accelerated by low dosages of narcotic drugs, but inhibited by high dosages.[40]

Free acetylcholine was reported in the spinal fluid in patients with epilepsy.[10,63] Of 56 epileptic patients, 44 demonstrated free acetylcholine in quantities of 0.02 to 5.0 gamma/100cc with an average of 1.0 gamma/100cc. Acetylcholine levels were related to the frequency of seizures, the extent of electroencephalographic abnormality, and to the time since

the last seizure but bore no relation to medication, type of epilepsy or level of cholinesterase activity. Elliott *et al.* also noted free acetylcholine in the spinal fluid in concentrations up to 3 gamma/100cc after pentylenetetrazol (Metrazol) convulsions.[16]

Tower and McEachern viewed the increased acetylcholine as a by-product of the seizure and not causal.[63] Studying the hypothesis that seizures were induced by the accumulation of acetylcholine, Torda measured the level of acetylcholine in brain tissue after pentylenetetrazol convulsions. She noted a rise in the acetylcholine content of brain before and a fall during the convulsion. Below certain levels of acetylcholine, convulsions failed to occur. She suggested that the fall in tissue acetylcholine during a convulsion was due to the inhibition of acetylcholine synthesis by increased concentrations of metabolites such as ammonium ions.[61,62]

Giarman and Pepeu also measured changes in central nervous system acetylcholine following various stimulants.[29] Only after methacholine and 3, 5-dimethylbutylethyl-barbiturate was there a significant change in the acetylcholine level. They noted a decrease in association with induced convulsions. With other drugs which they classified as stimulants (LSD, iproniazid, iproniazid + hydroxytryptophan, and iproniazid + DOPA) there were no changes in the acetylcholine level. They concluded that

despite intense excitation produced by these compounds, there were no changes in acetylcholine levels unless these were accompanied by convulsions. (The differences in observations between these observers and Cone *et al.* and Tower and McEachern may be related to the differences in methods of biochemical measurements, for the latter measured changes reflecting free acetylcholine only, while Giarman and Pepeu measured total acetylcholine including bound and free forms of acetylcholine.[40]).

These studies suggest that spontaneous or induced seizures are accompanied by an increase in intercellular free acetylcholine liberated from its bound form which may be reflected in the spinal fluid. Cerebral activity and seizures enhance acetylcholine destruction, lowering tissue levels of acetylcholine, while sleep and anesthesia augment acetylcholine production increasing tissue levels.

Central Nervous System Cholinesterases. Tower and McEachern also measured spinal fluid cholinesterase activity.[63,64,65] By reporting cholinesterase activity as a ratio of the rate of hydrolysis with two substrates compared to an acetylcholine substrate, acetylcholinesterase/acetylcholine and butyrylcholinesterase/acetylcholine ratios are derived. Normal cerebrospinal fluid contains these esterases in the ratio of 33:17.

In patients with head trauma, Tower and McEachern reported an inversion of the cholinesterases with an increase in the butyrylcholinesterase of the spinal fluid and a decrease in acetylcholinesterase activity. The extent of the cholinesterase reversal was related to the severity of trauma and to the degree of EEG abnormality. A similar reversal was observed in patients undergoing convulsive therapy.

In patients with elevated spinal fluid acetylcholine after spontaneous seizures, however, no change in the ratio of cholinesterases or total cholinesterase activity was found.

Changes in cholinesterase activity may be related to changes in cell membrane permeability. Acetylcholinesterase is found in highest concentration in the central nervous system while butyrylcholinesterase predominates in other tissues, especially blood serum. With increased cerebral acetylcholine, vasodilation and increased cellular permeability may be predicted, with vascular fluid transudation varying with the extent and duration of the vasodilation.[35] Spiegel, Spiegel-Adolf, and their co-workers demonstrated such permeability changes and increased conductivity of the tissues associated with the appearance of various ions (as potassium and phosphate) in the spinal fluid following electrically induced convulsions.[54-58] Such non-electrolytes as nucleic-acid splitting enzymes also increased.

Changes in cellular permeability may be the basis for the high concentrations of acetylcholine and increased concentrations of butyrylcholinesterase after induced seizures or head trauma.[65]

That changes in cholinesterases may be large and measurable is suggested by the recent demonstrations that neural stimulation and learning produces changes in brain weight and acetylcholinesterase activity.[37,49] Following these reports, Pryor and Otis studied the effects of repeated induced seizures in Wistar rats.[43] After as little as 4 weeks they observed increases in brain weight and in acetylcholinesterase activity which was related to decrements in behavioral performance.

The persistance of acetylcholine in spinal fluid after head trauma and after seizures despite increased cholinesterase activity may be related to the sensitivity of the acetylcholine-acetylcholinesterase system to concentration relationships.[8,41,65] At "physiologic" concentrations, hydrolysis of acetylcholine is rapid (3-4 microseconds) but at higher and lower concentrations, the activity falls off quickly. In contrast, the butyrylcholinesterase-acetylcholine relationship is non-specific and the rate of hydrolysis increases with increased concentration.

These relationships relate to theories of the induction of seizures. While the usual concentrations of acetylcholine at cell membranes are destroyed by the specific activity of acetylcholinesterase in a few microseconds, an excessive concentration following excitation may exceed its rate of

hydrolysis. The seizure threshold may be reached and a seizure induced, with the seizure itself adding to the amount of free acetylcholine. Increased acetylcholine affects vascular and cellular permeability altering the concentrations of various ions, including butyrylcholinesterase in tissues and in the cerebrospinal fluid. Through the activity of this esterase, though of low efficiency and depending on concentration kinetics. acetylcholine is reduced in tissues to levels for the more direct action of acetylcholinesterase.

Cholinesterases appears in the spinal fluid as a reflection of their increase in intercellular fluids resulting from changes in cell membrane permeability accompanying increased acetylcholine.

EEG Hypersynchrony and Induced Convulsions. The significance of high voltage EEG slow wave activity for the convulsive therapy process has been repeatedly described.[22,23,50,51] In the usual course of convulsive therapy, inter-treatment electroencephalograms record progressive increases in amplitude and in theta activity and a reduction in beta activity. As treatment continues, delta activity appears in bursts and eventually is the dominant activity in all leads. These changes are directly related to the number and rate of induced convulsions, and is not specific for a method of induction. While some relationships to type of electrical current has been observed, all seizure inducing methods — electrical, intravenous chemical or inhalant — exhibit the same type of EEG pattern changes.[21,22,23,30]

The early appearance of high degree hypersynchrony and its persistence throughout a treatment course has been found to be prerequisite to improvement. Both the electrographic and the behavioral changes of induced convulsions are transiently reversed by the acute administration of experimental anticholinergic compounds.[19,20] The intravenoue injection of diethazine, benactyzine, the piperidylbenzilates JB-318, JB-336 and JB-329 (Ditran), and WIN-2299 induced EEG desynchronization in psychiatric subjects. These EEG changes were associated with behavioral alerting, anxiety, tremors, illusions and hallucinations. In patients who had recently received electroconvulsive therapy there was a reduction in slow wave activity and a reversal of euphoria, denial and confusion. Atropine, in low doses, was also associated with EEG desynchronization accompanied by tachycardia, nervousness and tension. At higher dosages, hypersynchronous slow waves followed by lower voltage, poorly organized delta activity with superimposed beta activity was accompanied by progressive confusion and disorientation.

The effect of anticholinergic drugs on the slow wave activity of convulsive therapy was also assessed by the chronic administration of atropine (5 mgm per day) and scopolamine (1-3 mg) during the weeks of treatment. The amount of EEG slowing was significantly less than in a control group.[66] The samples were too small for a clinical correlation but the data is consistent

with blocking of the clinical effects of electroconvulsive therapy. Marked improvement was reported in 2 of 7 atropine-treated, none of 5 scopolamine-treated and in 4 of the 6 controls receiving unmodified ECT. This study was not replicated by the authors who suggest that dosage factors or population changes may have contributed to the different results in a second study.[34]

As in cerebral trauma, the electrographic changes of induced convulsions may be modified by the administration of anticholinergic drugs suggesting that increased amounts of acetylcholine or increased cholinergic receptivity is associated with the high voltage slow wave activity.

Acetylcholine and Induced Convulsions. Despite a constant application of treatments, however, there is great variability in the time of appearance, the duration, amount, and sensitivity to modification by alerting, hyperventilation and barbiturates of the electrographic slow wave activity in psychiatric populations.[30] These differences relate to differences in central cholinergic activity. The failure of certain patients to develop hypersynchrony may be associated with the absence of free acetylcholine and with minimal changes in cerebral function,

thus precluding a clinical response to induced convulsions. Tower and McEachern in their study of craniocerebral trauma, included observations of six psychiatric patients undergoing convulsive therapy.[63] Studying the patients after 3-7 treatments they reported free spinal fluid acetylcholine in two patients; and an increase in butyrylcholinesterase and a decrease in acetylcholinesterase with a reversal of the ratio of cholinesterases in five of the six patients. Concerning the one patient in the series who failed to show either free acetylcholine or a cholinesterase ratio reversal in the spinal fluid, the authors stated: "It is interesting that this patient was the only one of the six to show no response to treatment." From these observations they concluded that the spinal fluid changes in induced convulsions were more like those of craniocerebral trauma than those of spontaneous epilepsy.

Other evidence of alterations in the permeability barrier may be seen in the demonstrations of an increased concentration of cocaine in brain tissues three days after a series of 12 induced convulsions.[1] The change in concentration of this large molecule, ordinarily absent in brain tissue, was associated with the appearance of hypersynchrony (delta bursts) in the electroencephalogram.

From these observations we would conclude that induced convulsions, like craniocerebral trauma and spontaneous seizures, are associated with an increase in free acetylcholine in intercellular fluids, altering cerebral permeability and enhancing the appearance of cholinesterases. The level of free acetylcholine is maintained by repeated induced seizures. EEG hypersynchrony is one reflection of altered levels of acetylcholine and the altered permeability of electrolytes and other substances, including cholinesterases. The changes in intercellular electrolytes, including acetylcholine, provide the biochemical substrate for the persistent behavioral changes and EEG hypersynchrony following induced convulsions.

An application of these conclusions is seen in the studies of the prediction of the convulsive therapy response and the classification of psychoses.

Cholinesterases and the Classification of Psychoses. Funkenstein *et al.* reported a relationship between the blood pressure response to methacholine and the clinical response to convulsive therapy.[25-27] Immediately after the injection of methacholine the blood pressure falls, usually returning to the baseline within 5-20 minutes. A return within 5 minutes places the patients in Groups I, II or III; while a return after 20 minutes places the patient in Groups VI and VII.

Group I and Group II-III have a 9% and a 35% recovery rate, respectively, while Group VI and Group VII subjects have 89% and 97% recovery rates to induced convulsions.[27] Group I to III reactors may be looked upon as patients in whom methacholine is rapidly hydrolyzed; while Groups VI and VII have a slow hydrolysis rate. (The response to injected epinephrine was suggested as a second criteria in the classification, but is of limited discriminating value.[48]) While we have no biochemical explanation for the differences in the metabolism of methacholine in these psychiatric groups, it is possible that the blood and tissue cholinesterase activity levels of Groups I-III is high while that of Groups VI-VII is low compared to general psychiatric populations.

The differences in blood cholinesterase levels in normal and mentally ill subjects have been extensively studied. Despite differences in methods,[4,5] elevated cholinesterase levels compared to normal populations have been reported for depressive subjects,[44,46,47,52] schizophrenic subjects[14,28,53] and a mixed psychiatric populations.[42] Alpern reported lowered cholinesterase levels in schizophrenic subjects.[2] While these studies appear inconclusive, they provide data that the variations in blood cholinesterase levels are generally greater and frequently elevated in the mentally ill. Negative reports include the failure by Ellman and Callaway[17] to confirm Rubin's study; and Altschule's review of the data suggesting

no abnormality of cholinesterase levels in the mentally ill.[3]

These studies suggest that cholinergic measures may play a significant role in the therapeutic response to convulsive therapy and in the pathogenesis of psychoses.

CONCLUSIONS

This review summarizes some of the available data suggesting that cholinergic mechanisms may be central to the convulsive therapy process. Induced convulsions are associated with cerebral vasodilation and increased cellular permeability, followed by the appearance of increased amounts of enzymes and electrolytes in intercellular and cerebrospinal fluids. The increase in acetylcholine, vasodilation and increased permeability appear as interrelated phenomena associated with trauma, seizures and induced convulsions.

These biochemical changes accompany increased electrical hypersynchrony which is recorded as EEG slow wave activity in scalp electrodes and which can be modified by the acute and chronic administration of anticholinergic drugs as atropine, benactyzine, diethazine, procyclidine and various piperidyl-benzilates.

In these regards, induced convulsions are more similar to cerebral trauma than to spontaneous seizures.

The changes in cerebral biochemistry alter cellular activity sufficiently to affect consciousness and the behavior of subjects. Failure to induce persistent biochemical changes, including the concentration of acetylcholine, results in failure to produce behavioral change.

There is, as yet, no consistent evidence for differences in the sensitivity or dependence of populations on cholinergic mechanisms. Differences in the rate of development of cerebral changes to the same number and frequency of induced convulsions and classifications of the mentally ill based on the blood pressure response to methacholine suggest, however, that such differences may be significant in the pathogenesis of different psychoses.

REFERENCES

1. Aird, R. B., Strait, L. A., Pace, J. W., Hrenoff, M. K. and Bowditch, S. C. Neurophysiological effects of electrically induced convulsions. Arch. Neurol. Psychiat., *75*: 371-378, 1956.

2. Alpern, D. O. Aktivnost kholinesterasy i kholinergicheskaya reaktsiya krovi pri shizofrenii. [Cholinesterase activity and cholinergic reaction of the blood in schizophrenia.] Fiziol. Zh., *4*: 87-90, 1956.

3. Altschule, M. D. Vitamins, enzyme systems, and trace elements. *Bodily Physiology in Mental and Emotional Disorders*, pp. 169-172. Grune & Stratton, New York, 1953.

4. Augustinsson, K.-B. The normal variation of human blood cholinesterase activity. Acta Physiol. Scand., *35*: 40-52, 1955.

5. Augustinsson, K.-B. Assay methods for cholinesterase. In D. Glick, ed., *Methods of Biochemical Analysis*, vol. 5, pp. 1-63. Interscience Publishers, New York, 1957.

6. Bornstein, M. D. Presence and action of acetylcholine in experimental brain trauma. J. Neurophysiol., *9*: 349-366, 1946.

7. Brenner, C. and Merritt, H. H. Effect of certain choline derivatives on electrical activity of the cortex. Arch. Neurol. Psychiat., *48*: 382-395, 1942.

8. Burgen, A. S. V. and MacIntosh, F. C. The physiological significance of acetylcholine. In K. A. C. Elliot, I. H. Page and J. H. Quastel, eds., *Neurochemistry*, pp. 374-375. Thomas, Springfield, Illinois, 1955.

9. Chatfield, P. O. and Dempsey, E. W. Effects of prostigmine and acetylcholine on cortical potentials. Amer. J. Physiol., *135*: 633-640, 1942.

10. Cone, W. V., Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity in epilepsy. J.A.M.A., *73*: 59-63, 1948.

11. Crossland, J. and Merrick, A. J. The effect of anaesthesia on the acetylcholine content of the brain. J. Physiol., *125*: 56-66, 1954.

12. Dale, H. H. The action of certain esters and ethers of choline, and their relation to muscarine. J. Pharmacol. Exp. Ther., *6*: 147, 1914.

13. Denisenko, P. P. Pharmacological blocking of central cholinoreactive systems and the possibilities of its therapeutic application. In G. B. Koelle, W. W. Douglas and A. Carlsson, eds., *Pharmacology of Cholinergic and Adrenergic Transmission*. Vol. 3 of H. Rascova, general ed., *Proceedings of the Second International Pharmacological Meeting*, pp. 147-152. Macmillan, New York, 1965.

14. Early, D. F., Hemphill, R. E., Reiss, M. and Brummel, E. Investigations into cholinesterase levels in serum and cerebrospinal fluid of psychotic patients. Biochem. J., *45*: 552-556, 1949.

15. Eccles, J. C. *The Physiology of Synapses*, 316 pp. Academic Press, New York, 1964.

16. Elliott, K. A. C., Swank, R. L. and Henderson, N. Effects of anesthetics and convulsants on acetylcholine content of brain. Amer. J. Physiol., *162*: 469-474, 1950.

17. Ellman, G. L. and Callaway, E. Erythrocyte cholinesterase-levels in mental patients. Nature (London), *192*: 1216, 1961.

18. Fink, M. A unified theory of the action of physiodynamic therapies. J. Hillside Hosp. (Glen Oaks), *6*: 197-206, 1957.

19. Fink, M. Effect of anticholinergic agent, diethazine, on EEG and behavior: significance for theory of convulsive therapy. Arch. Neurol. Psychiat., *80*: 380-387, 1958.

20. Fink, M. Effect of anticholinergic compounds on post convulsive electroencephalogram and behavior of psychiatric patients. Electroenceph. Clin. Neurophysiol., *12* (2): 359-369, 1960.

21. Fink, M. The mode of action of convulsive therapy: the neurophysiologic-adaptive view. J. Neuropsychiat., *3*: 231-233, 1962.

22. Fink, M. and Kahn, R. L. Quantitative studies of slow wave activity following electroshock. Electroenceph. Clin. Neurophysiol., *8*: 158, 1956.

23. Fink, M., Kahn, R. L., Karp, E., Pollack, M., Green, M. A. Alan, B. and Lefkowits, H. J. Inhalant-induced convulsions. Arch. Gen. Psychiat. (Chicago), *4*: 259-266, 1961.

24. Freedman, A. M., Bales, P. D., Willis, A. and Himwich, H. E. Experimental production of electrical major convulsive patterns. Amer. J. Physiol., *156*: 117-124, 1949.

25. Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic nervous system changes following electric shock treatment. J. Nerv. Ment. Dis., *108*: 409-422, 1948.

26. Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic changes paralleling psychologic changes in mentally ill patients. J. Nerv. Ment. Dis., *114*: 1-18, 1951.

27. Funkenstein, D. H., Greenblatt, M. and Solomon, H. C. Autonomic nervous system test of prognostic significance in relation to electroshock treatment. Psychosom. Med., *14*: 347-362, 1952.

28. Gal, E. M. Cholinesterase activity of whole blood from healthy and schizophrenic individuals. Nature (London), *198*: 1118-1119, 1963.

29. Giarman, M. J. and Pepeu, G. Drug-induced changes in brain acetylcholine. Brit. J. Pharmacol., *19*: 226-234, 1962.

30. Green, M. A. Significance of individual variability in EEG response to electroshock. J. Hillside Hosp. (Glen Oaks), *6*: 229-240, 1957.

31. Hampson, J. L., Essig, C. F., McCauley, A. and Himwich, H. E. Effects of di-isopropyl fluorophosphate (DFP) on electroencephalogram and cholinesterase activity. Electroenceph. Clin. Neurophysiol., *2*: 41-48, 1950.

32. Himwich, H. E., Essig, C. F., Hampson, J. L., Bales, P. D. and Freedman, A. M. Effect of trimethadione (Tridione) and other drugs on convulsions caused by di-isopropyl fluorophosphate (DFP). Amer. J. Psychiat., *106*: 816-820, 1950.

33. Jenkner, F. L. and Lechner, H. The effect of Diparcol on the electroencephalogram in the normal subject and in those with cerebral trauma. Electroenceph. Clin. Neurophysiol., *7*: 303-305, 1955.

34. Johnson, L. C., Ulett, G. A., Johnson, M., Smith, K. and Sines, J. O. Electroconvulsive therapy (with and without atropine); effect on electronically analyzed electroencephalogram. Arch. Gen. Psychiat. (Chicago), *2*: 324-336, 1960.

35. Kabat, E. A., Glusman, M. and Knaub, V. Quantitative estimation of the albumin and gamma globulin in normal and pathologic cerebrospinal fluid by immunochemical methods. Amer. J. Med., *4*: 653-662, 1948.

36. Kovach, A. G. B., Fonyo, A. and Halmagyi, M. Acetylcholine content of the brain in traumatic shock. Acta Physiol. Acad. Sci. Hung., *13*: 1-4, 1957.

37. Krech, D., Rosenzweig, M. R. and Bennett, E. L. Effects of environmental complexity and training on brain chemistry. J. Comp. Physiol. Psychol., *53*: 509-519, 1960.

38. Loewi, O. Über humorale Übertragbarkeit der Herznervenwirkung. Arch. Ges. Physiol., *189*: 239-242, 1921.

39. Maynert, E. W. and Buck, E. G. Effects of C.N.S. depressants on brain acetylcholine. Pharmacologist, *6*: 191, 1964.

40. McLennan, H. and Elliott, K. A. C. Effects of convulsant and narcotic drugs on acetylcholine synthesis. J. Pharmacol. Exp. Ther., *103*: 35-43, 1951.

41. Nachmansohn, D. and Rothenberg, M. A. Studies on cholinesterase: on specificity of enzymes in nerve tissue. J. Biol. Chem., *158*: 653-666, 1945.

42. Plum, C. M. Study of cholinesterase activity in nervous and mental disorders. Clin. Chem., *6*: 332-340, 1960.

43. Pryor, G. T. and Otis, L. S. Effects of chronic administration of electroconvulsive shock on behavior, brain weight and brain chemistry. Presented at the American Association for the Advancement of Science, Berkeley, 1965.

44. Ravin, H. A. and Altschule, M. D. Serum cholinesterase activity in mental disease. Arch. Neurol. Psychiat., *58*: 645-650, 1952.

45. Richter, D. and Crossland, J. Variation in acetylcholine content of the brain with physiological state. Amer. J. Physiol., *159*: 247-255, 1949.

46. Richter, D. and Lee, M. Serum choline esterase and anxiety. J. Ment. Sci., *88*: 428-434, 1942.

47. Richter, D. and Lee, M. Serum choline esterase and depression. J. Ment. Sci., *88*: 435-439, 1942.

48. Rose, J. T. The Funkenstein test - a review of the literature. Acta Psychiat. Scand., *38*: 124-153, 1962.

49. Rosenzweig, M. R., Krech, D., Bennett, E. L. and Diamond, M. C. Effects of environmental complexity and training on brain chemistry and anatomy: a replication and extension. J. Comp. Physiol. Psychol., 55: 429-437, 1962.

50. Roth, M. Changes in the EEG under barbiturate anaesthesia produced by electro-convulsive treatment and their significance for the theory of ECT action. Electroenceph. Clin. Neurophysiol., *3*: 261-280, 1951.

51. Roth, M., Kay, D. W. K., Shaw, J. and Green, J. Prognosis and Pentothal induced electroencephalographic changes in electro-convulsive treatment. Electroenceph. Clin. Neurophysiol., *9*: 225-237, 1957.

52. Rowntree, D. W., Nevin, S. and Wilson, A. The effects of diisopropylfluorophosphonate in schizophrenia and manic depressive psychosis. J. Neurol. Neurosurg. Psychiat., *13*: 47-62, 1950.

53. Rubin, L. S. Acetylcholine hydrolysis in psychiatric patients. Science, *128*: 254-255, 1958.

54. Spiegel, E. A. and Spiegel-Adolf, M. Permeability changes in the brain induced by Metrazol and insulin convulsions. J. Nerv. Ment. Dis., *93*: 750-755, 1941.

55. Spiegel, E. A. and Spiegel-Adolf, M. Physicochemical effects of electrically induced convulsions (cerebrospinal fluid studies). Trans. Amer. Neurol. Ass., *70*: 130-132, 1944.

56. Spiegel, E. A. and Spiegel-Adolf, M. Physiological and physicochemical mechanisms in electroshock treatment. Confin. Neurol., *13*: 38-63, 1953.

57. Spiegel, E. A., Spiegel-Adolf, M. and Henry, G. Physicochemical changes in the brain accompanying electrically induced convulsive discharges. Trans. Amer. Neurol. Ass., *68*: 174, 1942.

58. Spiegel-Adolf, M., Wilcox, P. H. and Spiegel, E. A. Cerebrospinal fluid changes in electroshock treatment of psychoses. Amer. J. Psychiat., *104*: 697-706, 1948.

59. Stone, W. E. The role of acetylcholine in brain metabolism and function. Amer. J. Phys. Med., *36*: 222-255, 1957.

60. Tobias, J. M., Lipton, M. A. and Lepinat, A. A. Effect of anesthetics and convulsants on brain acetylcholine content. Proc. Soc. Exp. Biol. Med., *61*: 51-54, 1946.

61. Torda, C. Effect of convulsion inducing agents on the acetylcholine content of the brain. Amer. J. Physiol., *173*: 179-183, 1953.

62. Torda, C. Effects of single injection of corticotropin (ACTH) on ammonium ion and acetylcholine content of brain. Amer. J. Physiol., *173*: 176-178, 1953.

63. Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity. I. Cholinesterase patterns and acetylcholine in the cerebrospinal fluids of patients with craniocerebral trauma. Canad. J. Research, Sect. E, *27*: 105-119, 1949.

64. Tower, D. B. and McEachern, D. The content and characterization of cholinesterases in human cerebrospinal fluids. Canad. J. Research, Sect. E, *27*: 132-145, 1949.

65. Tower, D. B. and McEachern, D. Acetylcholine and neuronal activity. II. Acetylcholine and cholinesterase activity in the cerebrospinal fluids of patients with epilepsy. Canad. J. Research, Sect. E, *27*: 120-131, 1949.

66. Ulett, G. A. and Johnson, M. W. Effect of atropine and scopolamine upon electroencephalographic changes induced by electro-convulsive therapy. Electroenceph. Clin. Neurophysiol., *9*: 217-224, 1957.

67. Ward, A. A., Jr. Atropine in the treatment of closed head injury. J. Neurosurg., *7*: 398-402, 1950.

68. Wescoe, W. C., Green, R. E., McNamara, B. P. and Krop, S. The influence of atropine and scopolamine on the central effects of DFP. J. Pharmacol. Exp. Ther., *92*: 63-72, 1948.

Prepared for the Bio Sciences Information Exchange.

Not for publication or publication reference.

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

NOTICE OF RESEARCH PROJECT

PROJECT NO. (Do not use this space)
B-641 (C2)

NEURO (2)

SUBMITTED TO: Public Health Service, National Institutes of Health, Div. of Research Grants, Bethesda 14, Md.

TITLE OF PROJECT:

"Acetylcholine and Serotonin in Spinal Fluid and Brain of Head Injuries"

Give names, departments, and official titles of PRINCIPAL INVESTIGATORS and ALL OTHER PROFESSIONAL PERSONNEL engaged on the project.

Ernest Sachs, Jr., M.D., Neurosurgeon, Hitchcock Clinic; Instructor in Neurosurgery, Dartmouth Medical School

John P. Davison, Ph.D., Assistant Professor in the Physiological Sciences, Dartmouth Medical School

NAME AND ADDRESS OF APPLICANT INSTITUTION:

Hitchcock Foundation
Hanover, New Hampshire

SUMMARY OF PROPOSED WORK — (200 words or less — Omit Confidential data.)

In the Bio Sciences Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in medical and related fields and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

A quantitative study of the acetylcholine content of spinal fluid in head injuries, utilizing the bio-assay method and Venus Mercenaria, has been conducted during the past two years. The study indicates the presence of acetylcholine, serotonin, and other unidentified substances in the period immediately following head injury. The proposed study is an extension of this work to brain tissue as well as spinal fluid, and will utilize in addition chemical analysis for these same substances (spectrophotofluorometer).

Submitted for period
beginning - September 1956

SIGNATURE OF PRINCIPAL INVESTIGATOR Ernest Sachs, Jr.

Identify the Professional School (medical, dental, public health, graduate, or other) with which this project should be identified:

SCHOOL Hitchcock Foundation - Dartmouth Med. School

INVESTIGATOR — DO NOT USE THIS SPACE

12/17/56

SPECIFIC COLORIMETRIC DETERMINATION OF CHOLINESTERASE ACTIVITY IN BLOOD AND SPINAL FLUID[1]

- Harry Goldenberg and Vivian Goldenberg

Abstract of a paper prepared for presentation at the Meeting-in-Miniature of the Metropolitan - Long Island Subsection, New York Section, American Chemical Society, Brooklyn, N.Y., February 15, 1957.

A sensitive new colorimetric method is reported for the determination of cholinesterase activity. Though designed expressly for studies of the effect of lysergic acid diethylamide (LSD) and electroshock therapy on true cholinesterase in spinal fluid, the procedure is equally applicable to the analysis of serum, red blood cells, saliva, urine and other biological fluids. Only 0.3 ml. of spinal fluid or 0.001 ml. of serum is required for a determination.

Acetylthiocholine is employed as substrate in Tris buffer, pH 8. Following incubation, the enzyme activity is terminated by adding 5% trichloroacetic acid, centrifuging, and decanting the mixture into a colorimeter tube. The thiocholine is measured by first adding ferric - copper reagent at pH 4 and then o-phenanthroline. The thiol group reduces the ferric to ferrous ion, which now reacts with o-phenanthroline to produce a reddish color with an absorption maximum at 510 mμ. Either true or pseudocholinesterase can be determined provided the other is not present. When both enzymes are present, as in spinal fluid, true cholinesterase is measured by adding "Astra 1397" (10-α-diethylaminopropionylphenothiazine hydrochloride), a selective inhibitor of pseudocholinesterase.

The normal ranges of values in various biological fluids are demonstrated and compared to the findings in patients with mental disease.

The analytical procedure is also now employed to study substances that modify cholinesterase action, such as LSD, which is a potent inhibitor of

pseudocholinesterase. For this purpose the acetylthiocholine - ferrous - phenanthroline system is superior to the one previously described[2] and can detect 0.1 microgram of LSD as determined by the decrease in the amount of thiocholine liberated by serum.

[1] This work was supported in part by Research Grant M-1305 from the National Institute of Mental Health, U.S. Public Health Service.

[2] Goldenberg, H., and Goldenberg, V., J. Hillside Hosp., 5, 246 (1956).

CHROMATOGRAPHIC, ELECTROPHORETIC, AND COLORIMETRIC PROCEDURES FOR THE PSYCHOTOMIMETIC AND PSYCHOTHERAPEUTIC DRUGS[1]

- Harry Goldenberg and Daniel L. White

Abstract of a paper prepared for presentation at the Meeting-in-Miniature of the Metropolitan - Long Island Subsection, New York Section, American Chemical Society, Brooklyn, N.Y., February 15, 1957.

Despite their widespread use in psychiatry, specific separative and analytical methods are not available for the psychotomimetic and psychotherapeutic drugs. Research along these lines is of particular interest in two areas:

1) Studies on the relationship between the incidence of hallucinations in schizophrenics and their excretion of atypical alkaloids possessing comparable properties to the psychotomimetic drugs (mescaline, lysergic acid diethylamide, bufotenin), and

2) Studies on the oxidative metabolism of aromatic groups such as are present in chlorpromazine, reserpine, and tryptophan. That there is a metabolic defect in schizophrenia involving oxidative enzymes may be imputed from scattered reports of the excessive excretion of diazo-reacting material in the urine from schizophrenics.

We wish to present a complete scheme for isolating and quantitating alkaloids from urine. The alkaloids are extracted from alkalinized urine with purified ethylene dichloride, transferred into dilute acid, and thence back into a small volume of ethylene dichloride. The solvent is evaporated down almost to dryness, then spotted out on filter paper. Separation of the components is effected either by descending chromatography using butanol-

[1] This work was supported in part by Research Grant M-1305 from the National Institute of Mental Health, U.S. Public Health Service.

acetic acid - water (4:1:1), or by electrophoresis in 1% $KHCO_3$. The position of each component is established with the appropriate spray (see below). At this point the corresponding sections are cut out from parallel paper strips, the compounds eluted with alcohol, the ethanol removed by evaporating in calibrated tubes, then methyl orange and ethylene dichloride are added with shaking. The intensity of color formed by the alkaloid - dye complex in the lower organic layer is read directly in a colorimeter at 430 mμ and compared to the appropriate standard.

Much information concerning the structure of each alkaloid may be gleaned from the manner in which they react on paper to 5 sprays, viz., ninhydrin, the Dragendorff reagent, diazotized p-nitroaniline, iodoplatinate, and dimethylaminobenzaldehyde. Data are presented for 16 drugs demonstrating the specific structural features required for a positive test with each spray. Each drug can be differentiated from the others on the basis of the indicated spot tests. Application of the methods to the urine of schizophrenics prior to and following drug therapy will be discussed.

12-6-57

Effect of Diethazine on EEG and Significance for Theory of Convulsion Therapy

In a previous report to this society we noted the relationship between the degree of induced delta activity during the course of therapy and the behavioral response to electroshock. Those patients, in whom high degrees of delta activity were induced early, and were sustained, manifested the greatest degrees of behavioral change, as well as a significantly greater percentage of improvement and recovery than those patients in whom only low degrees of delta activity were induced (Fink and Kahn, 1957).

In the past few years, a variety of reports relating changes in free(?) acetylcholine and cholinesterase in the spinal fluids of patients (Sachs, Ward) and animals (Barnstein, Tower and McEachern) following head trauma; the observations that cholinalytic agents may alter the EEG patterns induced by trauma (Barnstein, Ward, Jenkner-Lechner) and by electroshock (Ulett), led us to investigate the role of acetylcholine-cholinesterase metabolism in electroshock therapy.

In 1956 Ulett reported that atropine or schpolamine, when administered on a round the clock schedule blocked the appearance of the delta activity we have come to associate with electroshock therapy. Ulett noted, however,

that his patients suffered numerous undesirable side effects during these observations. Previously, Ward (1950) following the suggestion of Bornstein (1946) had noted that atropine altered both the EEG patterns and the neurologic signs induced in man by head trauma. Here, too, the side effects were marked. In 1953, Jenkner and Lechner reported that EEG and behavioral effects similar to atropine were achieved by diethazine administered in patients with head trauma. They also reported the effect of diethazine in normal subjects.

It is the purpose of this report to describe the effects of intravenous diethazine on the EEG of patients during electroshock therapy; and to relate ~~their~~ these findings to the present neurophysiologic-adaptive hypothesis of the mode of action of convulsive therapy.

Diethazine is a soluble phenothiazine compound with phamacologic properties similar to atropine. In experimental animals, Heymans et al (1949) have noted that diethazine blocks vagal slowing of the heart; suppresses the bradycardia, bronchospasm, salivation, and fasciculation and seizures induced by acetylcholine, DFP and pilocarpine; and induces dry mouth, mydriasis and hypotension.

Subjects: Twenty-two psychiatric patients, in various stages of electro-shock treatment in an open-ward, voluntary psychiatric hospital have been studied. In these ~~experimentas,~~ experiments, subjects were tested in the EEG laboratory. Following a routine EEG recording, diethazine was administered intravenously at the rate of 25 mgm per minute, for a total of 200 to 250 mgm, depending upon the behavioral effects. Prior to the drug administration, an unstructured historical interview and a structured questionnaire period were tape-recorded. Following drug administration, both EEG recording, and recorded interview periods were continued until the EEG record again manifested the pre-injection patterns on visual inspection.

Observations:

(a) Clinical: All subjects manifested spontaneous coughing initially; followed by a dryness of the mouth and a thickness of speech. They noted a feeling of lassitude and weakness of the extremities; soon followed by increased restlessness and difficulty in maintaining eyelid closure.

Psychiatric phenomena were clearly manifested in some subjects. In the rest period between 13 and 30 minutes after drug administration, six subjects spontan-

eously voiced feelings of unreality, visual and haptic illusions, and delusional thoughts about their illness, the setting of the test procedures or our identity. Such patterns were transient and had disappeared by the termination of the experiment, usually within three hours. In three subjects, increasing agitation and panic led to a cessation of the recording. Here, too, restitution of pre-injection behavior was apparent within three hours.

(b) In previous studies, we had noted the intimate relationship between changes in syntactic language patterns with alteration in cerebral function induced by electroshock. In subjects tested prior to electroshock, diethazine induced changes in syntactic pattern of an "alerting" variety. In subjects with delta activity, with clinical syntactic patterns indicative of an alteration in cerebral function, diethazine induced a transient disappearance or minimzation of such language patterns. The period of changes in language was concurrent with changes in electroencephalogram.

(c) <u>EEG Patterns</u>: In all records, there is a decrease in voltage and desynchronization of frequencies. There is a decrease in prominence of prevailing rhythms. In patients without delta activity (pre-electroshock)

this dechronization and voltage decrease is occasionally accompanied by the appearance of small amounts of low voltage 6-7 cps activity. These are demonstrated in Slides 1, and 2. The basic alpha rate does not appear to be altered. The build-up in voltages and appearance of slower frequencies with hyperventilation is blocked.

In patients with varying degrees of induced high voltage delta activity resulting from convulsive therapy, there is a decrease in voltages; both random and burst delta activity disappears; and irregular, low voltage alpha and beta frequencies become prominent. These changes are noted in Slides 3 and 4.

This change in records is manifest in all electroshock subjects. It appears during drug administration, and persists for one to three hours. Concurrent with electroencephalographic changes, are the behavioral and language patterns just described. With the recustitution of the pre-injection EEG patterns, the pre-injection behavioral and language patterns again appeared.

DISCUSSION:

These observations confirm the report of Jenkner and Lechner of the effects of diethazine in "normal" subjects. We also note that diethazine alters records ~~and~~ with electroshock induced delta activity in a fashion similar to atropine and scopolamine, as described by Ulett. Furthermore, these patterns are similar to the effect of these anti-cholinergic compounds in records following head trauma. In these subjects, intravenous diethazine caused immediate changes both in the EEG and in behavior. It is apparent, therefore, that it readily affects the central nervous system, and its duration of activity is most useful for experimental purposes.

The previously cited studies by numerous observers of nervous system effects of head trauma point to an intimate relationship between the degree of neurologic dysfunction, the degree of EEG alteration; and the level of free acetylcholine in the spinal fluid. The effect of atropine both on the EEG, and ~~the~~ concomitantly on behavior in subjects with head trauma lends further support to the significance of free acetylcholine as the biochemical basis for the observed EEG patterns. In these studies of diethazine and electroshock, the intimate relationship between EEG patterns and behavior

have been reported. We note the parallel to the observations in head trauma. On the bases of these observations, as well as studies of spinal fluid cholinesterase levels, (Tower and McEachern, Fink and Goldenberg), we would suggest that the biochemical substrate of the electroshock process is similar to that of head trauma. Electroshock may be looked upon as a controlled method of inducing cerebral dysfunction for its behavioral effect.

Previous studies have demonstrated that alteration in cerebral function provides the physiologic basis for the behavioral changes in electroshock (Fink and Kahn, 1957). Such alteration in cerebral function provides the milieu for a change in the organism's adaptation to his environment. All aspects of behavior, as perception, language, mood, recall, memory, affect, etc. undergo change, and provide the basis for the therapist's evaluation of improvement. The studies of diethazine amplify this neurophysiologic adaptive hypothesis of electroshock by suggesting the type of biochemical substrate that underlies both the physiologic and the behavioral changes.

Summary:

Diethazine, a patent anti-cholinergic compound, was experimentally introduced intravenously in psychiatric subjects in various stages of convulsive therapy.

Electroencephalograms manifested a desynchronization of frequencies, decrease in voltage, and observation of hyperventilation responses in records without prior delta activity. Records with delta activity showed similar changes with disappearance of delta burst activity.

Concomitant with the electrographic effects, behavioral and language patterns indicative of a reversal of the electroshock effect were observed.

It is concluded that:

(a) Diethazine is a patent anti-cholinergic compound that readily enters the central nervous system upon intravenous administration.

(b) The biodemical basis for EEG changes in electroshock is similar to that of head trauma; and

(c) The biochemical basis of the mode of action of convulsive therapy may lie in the acetylcholine-cholinesterase system.

Recent studies in these laboratories re-evaluating the role of cerebral changes in the mechanism of electroshock action, demonstrated that a necessary pre-requisite for change in behavior was the development of early and persistent signs of altered cerebral function of which electroencephalographic ~~abnormality~~ slowing (1) was a significant index. ~~The~~ Understanding of the significance of this observation remained unclear until the recent ~~re~~ report of Ulett describing the ability of ~~high dose atropine~~ premedication with high doses of atropine to prevent the appearance of the EEG delta abnormality (2). Following the suggestion of this report, we investigated the role of acetylcholine and cholinesterase in electroshock therapy. ~~as available in the medical literature~~.

The data amply demonstrates a ~~the [illegible]~~ close relationships between the degree, and ~~of clear~~ persistence of the electroencephalographic abnormality and the appearance of measurable quantities of free Acetylcholine in the CSF, as well as a reversal of the normal ratios of B-cholinesterase to Mecholyl-cholinesterase (3).

The role of acetylcholine in the transmission of nerve impulses has been a subject for study since the first description of the vagusstoff effects by Loewi (4). The arguments as to whether acetylcholine is the main or only agent ~~transmitter~~ of

(RICHTER & Crossland - 1949)

in the transmission of the nervous impulse ~~are~~ not of primary concern here. It is sufficient to note that acetylcholine is a normal constituent of nervous tissue, that it exists in a bound form and is liberated during the excitation process, that it is rapidly hydrolyzed through the specific action of cholinesterase, ~~and that the breakdown and~~ and as rapidly reconstituted by the choline-acetylase system (Richter + Crossland). ~~Furthermore~~, normal cerebrospinal fluid contains no free acetylcholine (6), despite the rapid breakdown of bound acetylcholine during periods of activity and excitement. ~~and~~ The cerebrospinal fluid normally has a definite level of cholinesterase activity, ~~The cholinesterase is~~ while principally of the "true" or nucholyl hydrolyzing ~~fast~~ type.

In the absence of free acetylcholine, and under the "normal" conditions described the electroencephalogram fails to show any consistent abnormality.

(a) Effect of Cranio-cerebral Trauma.: The earliest changes in this variable was described by Bannister (1946) in a classical study of the relation of EEG changes, degree of experimental head trauma, and levels of free acetylcholine in the C.S.F.

In a study of cats subjected to varying degrees of head trauma, Bernstein first showed that free acetylcholine appeared in the CSF within a few minutes, and persisted for varying periods up to 48 hours. There was a positive relation between the degree of trauma and the quantity of free ~~CSF~~ acetylcholine, which varied between 2.7 and 9.0 Gamma percent. Concomitant electroencephalogram ~~changes~~ ~~changes~~ demonstrated patterned changes. Initially, the record were high voltage fast activity, interpreted as an ~~cutaneous~~ neuronal discharge, only to be followed by a short period of flattening of all recorded electrical activity. These periods were followed by prolonged periods of high amplitude sharp waves in the delta frequencies.

Concomitant with the electroencephalographic changes, Bernstein further noted that behavioral manifestations also were correlated with degree of trauma; as well as the level of free acetylcholine and the EEG abnormality. ~~[illegible]~~ with highest levels of acetylcholine, he noted the greatest degree of EEG abnormality as well as the greatest severity of the alteration in consciousness, reflex changes or the appearance of seizures.

To further substantiate these observations, Bernstein ~~administered~~ applied acetylcholine to the exposed cat cerebral cortex. When the concentration of acetylcholine was 1 gamma per-cent or less, he observed high amplitude sharp waves of low frequency in the electroencephalogram. When the concentration was increased to 2 gamma per-cent, the electroencephalogram became flattened.

Tower and McEachern (1949a) repeated these studies in human cases of varying types of head trauma. In 112 patients free acetylcholine was found in the cerebrospinal fluid only in patients following head trauma, recent grand mal seizures and electroshock therapy. The free acetylcholine varied from 0.2 to 100 gamma per cent. In addition, Tower and McEachern assayed the cholinesterase activity of the spinal fluid. They noted a sharp rise in the nonspecific cholinesterase (benzoylcholine-splitting) fraction and a ~~[illegible]~~ drop in the specific cholinesterase (methacholine-splitting) fraction in the patients with head trauma and those following electroshock therapy. No such inversion was demonstrated in the ~~[illegible]~~ fluid ~~[illegible]~~ containing free acetylcholine following spontaneous seizures. These authors also conclude that the level of free acetylcholine varies directly with the

degree of cerebral damage; adding, however, ~~the note~~ that the degree of ~~[illegible]~~ reversal of the cholinesterase fraction is an even more sensitive indicator of cerebral damage. ~~Electroencephalograms were~~

In most of these subjects electroencephalograms were taken at varying intervals following trauma. ~~There~~ Here, too, as in Bornstein's experimental study, there was a direct correlation of the extent of EEG abnormality and the appearance of free acetylcholine in the cerebrospinal fluid

~~Thus, free acetylcholine has been found in the cerebrospinal fluid under the conditions of head trauma, electroshock therapy and seizures. A correlation between~~

Thus, we may conclude that craniocerebral trauma results in ~~the~~ the appearance of increased amounts of acetylcholine in the spinal fluid; and that ~~a correlation~~ a direct relation between the amount of acetylcholine ~~[illegible]~~ the degree and type of electroencephalographic abnormality and ~~the~~ clinical behavior.

post-traumatic

(6) Effect of Atropine on EEG and Behavior:

Following the studies of Bornstein and Tower and the Eatherly Ward (1950) administered large doses of atropine to patients in various stages following closed head injuries.

In his studies, Bornstein, administering 0.5-1.0 mg/kg. atropine, demonstrated a reversal of or a blocking of the EEG effects of trauma, depending on the relation of the dose to the trauma. Atropine also modified the behavioral and neurologic signs of trauma. ~~Earl (1950)~~ In the experimental condition of intracisternal acetylcholine, which induced EEG and clinical changes ~~not~~ similar to head trauma, Bornstein also demonstrated the blocking and reversing effect of atropine.

Ward (1950) applied these ideas to the treatment of human cases of closed head injury. In 20 patients with varying degrees of trauma he administered atropine subcutaneously in doses of 0.1 mg/kg. In selected cases he noted dramatic clinical improvement which was attributable to the atropine action. He also noted, in selected instances, ~~effects~~ reversal of the electroencephalographic effects of trauma by atropine. (* over)

More recently, ULETT ~~Ulett~~ (~~1956~~) and Johnson (1956)

(*) In the study of another anticholinergic drug, "DIPARCOL" (DIETHAZINE), Jenkner and Lechner (1955) report significant alterations in the post-traumatic electroencephalograms. A single intravenous dose in 40 instances ~~[illegible]~~ of abnormal electroencephalograms resulted in normalizing in 22 instances and marked improvement in six others. ~~with moderate [illegible] alteration in clinical behavior~~

dm →

demonstrated the same effect of peripheral atropine to block the occurrence of slow wave activity following electroshock therapy. This study suggests the possibility that the same biochemical reaction underlies the electroencephalographic abnormalities in lead trauma and in electroshock.

One report in the literature is at variance with these findings. In their experiments Brenner and Merrett (1947), applying topical acetylcholine in concentrations of 2½ to 10% to the exposed cortex of cats, noted no effect of intravenous atropine (1 mg/Kg) on the electroencephalographic changes. It is important to note however, that the concentrations of acetylcholine in these experiments were significantly higher than the topical applications (1-4 gamma%) and the intracisternal (0.2-10 gamma) injections of Bonnet-Gellhorn (1946). Brenner & Merrett, however, made no note of electroencephalographic effect similar to acetylcholine from mecholyl (acetyl-beta-methylcholine) and doryl (carbamylcholine), each in concentrations much lower than the acetylcholine concentrations.

they ascribed the increased effectiveness of these cholinergic drugs to their lack of penetration to cerebral cholinesterase.

A variety of experiments utilizing DFP (di-isopropyl fluorophosphate) - a compound with ~~an~~ irreversible anti-cholinesterase ~~effects~~ - demonstrated the development of high ~~voltage~~ amplitude rapid frequency waves similar to photic spike epileptiform, as well as lesser degrees of abnormality noted in post-traumatic states (Hunter et al, 1950; Freeman et al, [redacted] 1949; and Hampson et al, 1950). In these studies, too, the electroencephalographic effects were blocked by small doses of atropine.

In another laboratory study, Chatfield and Dempsey (1942) prepared exposed animal cortex with prostigmine and evoked electroencephalographic activity. The prior administration of atropine blocked the spiking, or if present, the abnormality could be eliminated by atropine.

Thus, from a variety of experimental and clinical studies, we may conclude that ~~an~~ electroencephalographic activity induced by acetylcholine, either as a result of

trauma, topical application or interference with
normal cerebral metabolism, can be blocked or
illuminated by absence.

(c) ~~Role~~ Role of Acetylcholine in ~~Cerebro-spinal Fluid~~ Seizures.

(c) Role of Cerebrospinal Fluid Acetylcholine in Seizures

~~The level of acetylcholine~~

Acetylcholine is normally present in ~~the~~ nervous tissue in a bound, inactive form. During periods of activity, the free acetylcholine is liberated at the cell membrane, where it is rapidly deactivated by ~~the~~ cholinesterase activity. The level of central nervous system acetylcholine is thus the resultant of the processes of synthesis, liberation and breakdown. It may be postulated, therefore, that the level and ~~free~~ that free during sleep and fast during activity. This hypothesis is ~~true~~ was demonstrated by Richter + Crowland (1949) and Elliott, Swanck and Henderson (1950) in animal experiments. By using bound and free assay methods, Richter and Crowland demonstrated that the anesthesia and sleep level of acetylcholine (measured as micrograms per mg brain tissue) was 300% higher than the last pregnane level. The difference in tissue levels is transient, however, as the resynthesis rate for acetylcholine in rat brain is high (1 gamma/gm/minute). Elliott et al ~~fe~~ confirmed

these observations. In addition, they ~~further~~ noted that after methacholine convulsions free acetylcholine was always demonstrable in the spinal fluid in concentrations up to 3 gamma per cent.

In spinal fluid studies in man, Cone Tower and McEachern (1948) and Tower and McEachern (1949 B) also demonstrated significant quantities of free acetylcholine ~~in~~ in patients with epilepsy. Of 56 epileptic patients, 77% (44) demonstrated measurable free acetylcholine in quantities of 0.02 to 5.0 gamma per cent, with an average of 1.0 gamma per cent. The acetylcholine level was directly related to the frequency of seizures; the extent of electroencephalographic abnormality; the ~~length of~~ relation of time of sampling to last seizure. It bore no relation to medication, type of epilepsy or level of Cholinesterase activity.

As to whether the acetylcholine appeared in the spinal fluid as a by-product of the convulsion, or whether the increase in ~~free~~ acetylcholine was a cause of the seizure, ~~is~~ is problematical. Tower & McEachern (1949B)

~~interpret their observations as suggesting~~

believe that the increased acetylcholine release is not due to the seizure itself but related to the basic process causing the seizure.

In a study of the hypothesis that the accumulation of acetylcholine is basic to the seizure process, Torda (1953), ~~in animals~~ reduced the ~~in which convulsions were induced~~ by metrazole - determined the level of acetylcholine in brain tissue before and during convulsions. She stated that convulsions are preceded by a rise in the acetylcholine content of tissue; ~~but~~ the content gradually falls during convulsions and that below certain levels convulsions failed to occur. Therefore she postulated a second factor which, in physostigmine convulsions was probably acetylcholine, but in electroshock ~~or tetanus toxin~~ seizure was not.

~~Further~~

She also concluded that the fall in tissue acetylcholine during a convulsion was due to inhibition of acetylcholine synthesis by increased concentration of metabolites as ammonium ions.

While considerable argument wages about

Is there a relationship between age and the activity, amount, or type of cerebral or other tissue cholinesterase?

8/28/56

8/28/56

On the Role of Acetylcholine and Cholinesterase in Electroshock Therapy

1. Introduction

EST dependent on delta abnormality - early - persistent

Recent review by Ulett → Atropine & delta abnormality & review of lit of ACH metabolism

2. Lit. a) Trauma -

1) Barnstein

2) Tower - the Teacher

EEG in trauma.

death Related to CSF free ACH - muscle choline

a) Effect of Atropine on EEG in trauma -

exp. ACH → Kennett → DFI - Hunkeler.

(Bloomquist has done before.)

b) Relation of ACH in CSF to present symptoms in EPI.

d -> Cholinesterase reversal in trauma.

d) CSF ACH in EST -

note about pts who did not response

Relation of EEG changes related to CSF ACH in trauma + EST.

c) diff between EEG in trauma & EPI is in cholinesterase. which changes in trauma, not EPI. ∴ EST in Trauma

3. Hypothetical relationships:

(I) (a) EST, either through the electrical activity directly or (produced) the grand mal, → free ACH

(c) Presence of free ACH → hyperexcitability of EEG → delta

(c) Absence of delta → absence of ACH → no change in cerebral function → no improvement

(II) ~~(d)~~ Why do some pts have normal delta?

(1) high Chol E → destroys ACH

(2) ~~Possible~~ Role of adrenaline metabolism.

(3) Compensatory cholinesterase rise may correct basic CNS error-

(III) Possible explanation for mecholyl test -

4) Studies to be undertaken -

a) Relation of cholinesterase activity of CSF and

1) age

2) dx

3) clinical reactivity

b) Free ACH and patterns of cholinesterase in CSF following EST; relation to EEG change, to clinical changes and to #, frequency, type of Rx

7/25/56

ACH in EST

1) EST increases free ACH in cells + CSF
2) Free ACH correlated with delta + spike activity of EEG.
3) Delta activity blocked by atropine (?)
4) In normal state, equilibrium exists

Bound ACH $\xrightarrow{\text{activity}}$ Free ACH $\xrightarrow{\text{ChoE}}$ Breakdown Prod.

Chol-acetylase + Substrate → NH_4^+

{ Bound ACH inc in sleep, anesthesia
" " dec " activity, convulsion

Free ACH ↑ c trauma, pre-convulsive, convulsion,

5) Adrenaline "low" concent augments ACH activity
augments action of prostigmine
but in "high" conc. will depress ACH activity

6) Synthesis rate of ACH is very rapid

7) Seizures: a) Minimum level of ACH required
b) induced by agents that raise ACH concent.
i.e. ACH, Mecholyl, Eserine, Prostigmine, DFP
c) Free ACH in CSF in EPI as Post Traumatic

7/25/56

8) Trauma - Chm. EEG correlates ~ free ACH is good
Also, same correlation ~ level of cholinesterase (true) -
Blocked by atropine
(note: "compensatory" increase in Cholinesterase noted
in pts ~ EPI ~ temporal focus)

9) Cholinesterase:
Levels vary ~ degree of trauma
Trauma inc B chol E factors (probably from outside CNS)

---

10) Possible relationships
a) Increased ACH provides milieu for sleep

Anxiety inc adren. ↓ sleep } equilibrium?
EST inc ACH ↑ sleep

Inc activity will reduce available ACH
Assuming free ACH is unavailable in cells.

c) If free ACH ↑, Ach cholinesterase ↑ - balance
is achieved between rate of free ACH production
by trauma + disappearance. In high conc of ACH,
Cholinesterase ineffective.

8/28/56

anxiety ⟶ Adr ↑

↑ Adr + Ach ⟶ (augmented) ⟶ inc. tension

Est ↓ tissue Ach — ↓ tension

↑ $\frac{Ads}{Ach}$ ratio changes - favors ↓

↑ free Ach — ∴ ↑ choline

which decreases tissue Ach ⟶ ↓ tension

---

1. l. differing relationships of $\begin{cases} Ach \\ Ads \end{cases}$ + MCS ↑

Perhaps what explains the change is the fact that at low Ach levels + high Adr levels, Ach is further inhibited — ∴ tension fades & accom- if Ach is the main factor again.

7/20/56

But atropine does not block CSF acetylcholine except in big doses — of Bremner + Merritt

10)

c) If atropine blocks AcH —

" EEG

it may block behavior

∴ OMS due to 1) trauma + 2) Free AcH altering cellular function (transmission of impulses)

note idea of Wiener-procedure

d) Since c̄ Depression, AcH appears in CSF more readily —

∴ destroyed less readily

? ∴ Cholinesterase activity is ↓ than in group w schiz

If trauma → inc CSF AcH → compensatory inc cholinesterase level

Could it be that inc Cholinesterase thereby corrects a basic biochemical defect?

[? relation of { adrenaline / anxiety } to AcH and cholinesterase

if anxiety ↗ Adr.

Adr + AcH = inc CNS activity = inc tension

AcH higher in depr because Cholinesterase is low

∴ when EST ↗ decreases (?) tissue AcH + inc Free AcH + inc Cholinesterase

E2
1

Neurohumoral factor in EST 6/26/56

(1) EST change result of EEG type changes

(2) (1) related to free ACH

(3) Bound ACH $\xrightarrow{\text{Trauma}}$ ACH (free)
$\xleftarrow{}$ Cholinesterase

If chol. Est is lacking or low, then
ACH (free) accum power -
∴ EEG changes "
∴ "improvement" - power.

(4) Mecholyl test.

(a) If mecholyl induces a fall in BP = ACH effect = low ~~Hight~~ Cholinesterase.
This latter may reflect age or
dx (i.e. - high in Schizy
Low in Inv.)

ACH

Could free ACH in CSF affect the brain stem centers
to diminish the outpouring of adrenalin -

The increase ACH leads to sleep - as it provides the cerebral
medium for sleep

Does tension, agitation → decrease CNS ACH?

7-11-56

Acetylcholine in EST

1) What is effect of ECT in ~~a~~ patients with myasthenia? (not available) | should be very different to induce CNS.- esp. c̄ EEG changes.

2) " " EEG effect of IV acetylcholine - [illegible]

3) " " " " " scopolamine } in normal
atropine }

4) What is relation of steroids to cholinesterase?

~~5) Behavioral effect of~~

5) What studies have been made of CSF or blood cholinesterase? Any following EST?

6) Relation of cholinesterase & Mg^{++} metabolism?

7) Does IV Acetylchol. or } → Convuls → CSF ACH?
IV metrazole }

i.e. does a peripheral seizure → CSF ACH

8)

Lab. of Exper. Psychiatry.