SUPPLEMENTUM 77

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and
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CEREBRAL CHANGES FOLLOWING ELECTRICALLY INDUCED CONVULSIONS

An Experimental Study on Cats

by

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S D G

To the Memory of my Father

and

to Ingeborg

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PREFACE

The present investigation was carried out between 1948 and 1952. The initial incitement was provided during an earlier collaboration with Professor Holger Hydén, then of the Institute for Medical Cell Research of Karolinska Institutet (Head: Professor T. Caspersson). I had originally hoped to study the problem with the ultra-violet microspectrographic techniques elaborated there. The work was therefore started at the aforementioned Institute. I found, however, that before such intensive methods could be applied, considerable work—necessitating the use of more extensive methods—was necessary, in order to select objects suitable for study from the neuropathological point of view. The investigation, of which an account is given in the following, was therefore continued at King Gustaf V's Research Institute (Head: Professor Nanna Svartz).

I received my training in psychiatry under Professor Torsten Sjögren, Head of the Psychiatric Clinic of Karolinska Institutet.

I take this opportunity of expressing my sincere gratitude to:

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During the course of my work I have derived considerable benefit and stimulation from my discussions with colleagues at the Institute for Medical Cell Research, King Gustaf V's Research Institute and the Psychiatric Clinic. I would like especially to thank Dr. Gunnar Moberger and Dr. Gunnar Holmberg.

I wish particularly to express my appreciation to the translator, Mrs Erica Odelberg, who with skill and unfailing regard for the subject matter rendered the Swedish manuscript into English.

Mrs Ingrid Åsberg carried out the histological preparation of the material; her technical ability was of invaluable assistance. The photomicrographs were taken by Mr Stig Hedman. Mr Olle Olson devoted great care to the experimental animals and also assisted at the operations. My warm thanks are due to all these persons, as well as to the entire staff of King Gustaf V's Research Institute.

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Stockholm, October 1952.

HANS HARTELIUS.

SCOPE OF THE INVESTIGATION

Cerletti & Bini (1938) were the first to use generalized epileptic seizures induced by electric shock as a therapeutic method in psychiatry. The method soon became widespread and is now presumably one of the most common forms of treatment in mental disorders, particularly in depressive states, in which it has been found to be of great value. It is therefore of prime interest to psychiatrists to ascertain whether its use is associated with evidence of damage to the brain, and, if so, the nature of such changes and, finally, whether or not the changes are reversible. The present investigation is an attempt to answer these questions by means of a special histological technique, a microscopical examination without previous knowledge of the origin of the specimens (unbiased examination), and the use of modern statistical methods. The experimental material consisted of cats subjected to electric convulsive treatment (abbreviated in the following to ECT). Untreated cats (controls) were used as comparative material.

ABBREVIATIONS AND SYMBOLS USED IN THE PRESENT STUDY

ECT = electric convulsive treatment.
C = control animals.
A, B, D, E = the respective groups of animals subjected to ECT (see page 29).
A_{24} = the index denotes the survival time in hours after the last ECT (8d = 8 days).
J = joule.
a.c. = alternating current.
m.a. = milliampere.
m. = monophasic.
d. = diphasic.
$\bar{x}$ = the arithmetical mean.
n = the number of values.
s = the calculated standard deviation.
t = Student's ratio.
p = the calculated value of the probability.
$\sim$ = denotes the difference between two groups of animals.

PART ONE

NEUROPATHOLOGICAL INVESTIGATIONS: SURVEY OF THE LITERATURE

CHAPTER 1

Introduction

a. LARGE DOSES OF CURRENT

Long before Cerletti & Bini started to use electricity to produce epileptic seizures for therapeutic purposes, numerous neuropathological studies were published on the effect of electric current on the brain. They were, however, made on a different basis to that relevant to investigations of changes associated with ECT and, without exception, dealt with higher strengths of current or other methods of application than those used clinically. This applies not only to the animal experiments but also to autopsy studies of persons electrocuted accidentally or legally.

Since the cerebral changes described are not comparable with those found in connexion with ECT, I shall not discuss the reports, but refer to such authors as URQUHART[107], MORRISON, WEEKS & COBB[73], JAFFÉ[50], PANSE[78], SPITZKA & RADASCH[101], LANGWORTHY[59] and HASSIN[40]. The interest of the aforementioned authors was focused mainly on the damage caused by the electric current and not on epileptic seizures such as they occur in ECT.

I shall also omit a discussion of the neuropathological changes observed in convulsions produced by other methods, such as pentrazol shock and insulin shock.

b. NEUROPATHOLOGICAL FINDINGS IN DEATHS FOLLOWING ECT (HUMAN AUTOPSY MATERIAL)

The death risk in ECT is very low. KOLB & VOGEL[55a], in their survey of 305 American hospitals, found a death rate of 0.06 per cent. IMPASTATO & ALMANSI[49a] found 0.08 per cent in their survey of the literature on ECT. The aforementioned figures refer to an early stage of electric shock therapy; with improved technique the risk appears to have decreased still further. Since it is a question of sporadic, extreme cases, the material as a whole is heterogeneous and each author described only a few cases or even a single one. Among those

who reported deaths are FAURBYE[27], DE CARO[14], EBAUGH *et al.*[23], ALPERS & HUGHES[2], RIESE[84], MEYER & TEARE[70], RIESE & FULTZ[85], WILL, REHFELDT & NEUMAN[110] and ZEMAN[114]. Concurrent severe somatic diseases, such as those of the cardio-vascular system, reported by the majority of authors make the interpretation of the possible cerebral findings more difficult.

The literature on cerebral changes in epilepsy is extensive. Reference is made to SCHOLZ's monograph[91] for a collocation up to 1951. It also contains a short summary of the experimental animal studies on cerebral changes in ECT. Scholz did not, however, analyze them closely since he considered the matter to lie beyond the scope of his publication.

In the following, I shall only comment on the literature dealing with experimental animal studies on the neuropathological changes associated with ECT.

CHAPTER 2

Earlier Experimental Studies on ECT *in Animals*

The most important data reported in earlier investigations are assembled in Table 1. A brief survey of the results is given in the following.

a. COLLOCATION OF EARLIER EXPERIMENTAL FINDINGS IN ANIMALS, CLASSIFIED ACCORDING TO THEIR NATURE

1. *Vascular changes*

a. CERLETTI & BINI[17]: vascular dilatation, some œdema, tortuosity of the capillaries, pigment bodies in the perivascular spaces, but no actual hæmorrhages.

b. ALPERS & HUGHES[3]: subarachnoidal hæmorrhages, small hæmorrhages in the brain parenchyma and, in one case, a hæmorrhagic infarction.

c. HEILBRUNN & WEIL[43]: localized hæmorrhages in the pia-arachnoid.

d. NEUBUERGER *et al.*[76]: vascular dilatation and small hæmorrhages.

e. LIDBECK[63]: isolated perivascular hæmorrhages and capillary thrombi.

f. ALEXANDER & LÖWENBACH[1]: with large strengths of current (1500 m.a.) transitory vasoconstriction; with still greater strengths of current (2000 m.a.) vaso-paralytic stasis.

g. FERRARO *et al.*[29]: dilatation of the pial vessels, congestion, perivascular pigment (possibly hæmotogenic), diapedesis, intimal reactions, œdema.

h. FERRARO & ROIZIN[30]: diapedesis, and petechial hæmorrhages after intensive treatment.

i. BJERNER, BROMAN & SWENSSON[8]: no hæmorrhages but some increase in the vascular permeability to trypan blue solutions.

No vascular changes

BARRERA *et al.*[5], FETTERMAN[31], GLOBUS *et al.*[36], WINKELMAN & MOORE[112], SIEKERT *et al.*[93] MASSERMAN & JACQUES[68] found hæmorrhages in the brain of both the control animals and those treated, but expressed the opinion that they were not to be ascribed to the shocks.

2. *Glial changes*

a. Cerletti & Bini[17]: progressive and regressive changes.

b. HEILBRUNN & LIEBERT[42]: slight swelling.

c. Alpers & Hughes[3]: reactive only in the vicinity of the hæmorrhages.

d. Heilbrunn & Weil[43]: reactive post-hæmorrhagic changes.

e. Neubuerger *et al.*[76]: scattered satellitosis and neuronophagia; slight proliferative changes in the glia and microglia cells.

f. Ferraro *et al.*[29]: slight reactive changes, satellitosis, neuronophagia.

g. Ferraro & Roizin[30]: hypertrophy and slight hyperplasia, particularly in the vicinity of the blood vessels.

No glial changes

Barrera *et al.*[5], Globus *et al.*[36], Lidbeck[63], Fetterman[31], Winkelman & Moore[112], Masserman & Jacques[68], Siekert *et al.*[93]

3. *Nerve cell changes*

Pyknosis: Cerletti & Bini[17], Barrera *et al.*[5], Neubuerger *et al.*[76], Lidbeck[63], Winkelman & Moore[112].

Tigrolysis and similar changes: Cerletti & Bini[17], Heilbrunn & Liebert[42], Barrera *et al.*[5], Neubuerger *et al.*[76], Lidbeck[63], Ferraro *et al.*[29 30]

No nerve cell changes

Alpers & Hughes[3], Fetterman[31], Heilbrunn & Weil[43] (only in the immediate vicinity of the hæmorrhages), Globus *et al.*[36], Winkelman & Moore[112], Masserman & Jacques[68], Alexander & Löwenbach[1] (with clinical strengths of current), Siekert *et al.*[93] It may be mentioned that Barrera *et al.*[5] also described slight nerve cell changes, but did not attach any importance to them since such changes were also observed in their control animals.

4. *Irreversible changes in the form of disappearance of nerve cells and areas of devastation*

Cerletti & Bini[17], Ferraro *et al.*[29], Ferraro & Roizin[30]: the authors nevertheless emphasized that the changes were mainly of the reversible type.

Alexander & Löwenbach[1]: irreversible changes only with very large strengths of current.

A study of the relevant literature reveals a considerable variation in the pathological findings, and the conclusions drawn from them by the different authors appear to be conflicting. Ferraro, Roizin & Helfand[29] made a survey of the literature up to 1946 and analyzed some of the reasons for which the observations differed in the respective investigations. They found a lack of conformity with respect to the following factors:

1. Strength of current.
2. Current flow time (from 0.1 seconds to several minutes).
3. Size of the electrodes (from less than one to several square centimetres).
4. Interval between the ECT's (from several per day to two weekly).
5. Number of ECT's (from single shocks to 100).
6. Species of experimental animals.
7. Diet of the experimental animals.

Taking into account the variations in the aforementioned factors, a number of the most conspicuous pathological changes could, with some degree of certainty, be ascribed to unsuitable techniques. The authors nevertheless considered that the conflicting statements could not be entirely explained on these grounds. They emphasized that attempts must be made to establish quantitative bases for judging the neuropathological observations.

In addition to the aforementioned factors, the points enumerated in the following should, in my opinion, be taken into consideration when setting up the experimental bases:

8. Method for sacrificing the animals.
9. Survival time between ECT's and death.
10. Fixation medium for the brain preparations.
11. Fixation method, immersion, perfusion.
12. Staining methods.
13. The use of control material and its treatment.
14. Age of the experimental animals and the controls.
15. Statements of other pathological phenomena, such as encephalitis.

The occurrence of artefacts is a matter of great importance; points 8, 10, 11 and 12 have a particular bearing on this question.

The *species* of experimental animal may be considered first. Experience has shown that *rabbits* are somewhat unsuitable. According to Heilbrunn & Weil[43], they only react with epileptic seizures when large doses of current (60—150 volts a.c. with a strength of current of 65—300 m.a. for 0.30—0.50 seconds) are used. Moreover, 16 out of their 28 animals died of paralysis, mainly affecting the hind legs and the urinary bladder, with ascending infection of the urinary tract. The aforementioned authors also observed hæmorrhages in the meninges and perivenous hæmorrhages basally in the cerebral parenchyma; according

to Ferraro *et al.*[29] these changes must presumably be ascribed to the high dosage of current.

SCHMITERLÖW & SILFVERSKIÖLD[90] studied the circulatory conditions associated with ECT in rabbits. They used 140 volts (50 periods) a.c. and a current flow time of 0.20—0.30 seconds. Such doses are sufficient to produce convulsions in man.

Bjerner, Broman & Swensson[8] did not state the size of the shock dose but reported the death of 1 out of their 4 rabbits during ECT.

Rats can scarcely be expected to provide reliable data regarding the neuropathological changes associated with ECT.

Monkeys were used by Barrera *et al.*[5], Ferraro *et al.*[29], Ferraro & Roizin[30] and Siekert *et al.*[93] The three first-mentioned authors used macacus rhesus and the last-mentioned macacus mulatta. In the same order, the shock doses were as follows: 70—135 volts a.c. for 0.10—0.15 seconds, 70—90 volts a.c. for 0.10—0.40 seconds, 90 volts a.c. for 0.30—0.50 seconds and 90 volts a.c. for 0.10—0.40 seconds; the agreement was thus good.

Dogs were chosen by Cerletti & Bini[17] (125 volts a.c. for 2—5 seconds), Neubuerger *et al.*[76] (80 volts a.c. for 0.15 seconds), Globus *et al.*[36] (electronarcosis) and Lidbeck[63] (unstated strength of current for 0.20—1.0 seconds). All these workers stated that the values only slightly exceeded the threshold value for epileptic seizures, but in the case of the first-mentioned authors the current flow time appears to be very high.

Cats were used by Alpers & Hughes[3] (110 volts a.c. for an unstated time), Winkelman & Moore[112] (70—80 volts for 0.10—1.0 seconds) and Masserman & Jacques[68] (30 volts a.c. for 5 seconds). — Cats were also used in the present investigation; I found that epileptic seizures could easily be produced with 55—70 volts a.c. and a current flow time of 0.20 seconds, although in the later stage of the experiments I used condensor discharges. The first-mentioned authors did not mention the current flow time but the voltage appears to be somewhat high.

Thus, when studying the conditions for producing epileptic seizures with electric current, monkeys, dogs and cats appear to be relatively suitable both for mutual comparisons and for comparisons with man.

Unless it is very large—or very small—in relationship to the head of the experimental animal, the *size of the electrodes* appears to be of minor importance. Many authors have omitted to state the size used. Relatively large electrodes (2 cm^2) were used by Heilbrunn & Weil[43] on rabbits, by Winkelman & Moore[112] (2.5 cm in diameter) on cats, Masserman & Jacques[68] (4 cm^2) on cats and Ferraro & Roizin[30] (2 cm^2 and 14 cm^2, respectively) on monkeys. The last-mentioned workers were unable to note any difference in the neuropathological findings when they made use of large or small electrodes, respectively.

A basic question is the *number of shocks* and the *interval* between them. The majority of workers have used methods resembling as closely as possible those in use clinically, *i.e.*, 2—6 treatments, most commonly 3, weekly. Cerletti & Bini[17], however, gave 1—2 daily and in another series, which is not comparable in the present connexion, multiple shocks (20—70 at intervals of 3—5 minutes). The variation in the number of shocks is appreciable, from 1 to 100, the most usual number being up to 20. This variation does not, however, suffice to explain the discrepancies in the findings of the different authors. This is apparent on a comparison between, on the one hand, Globus *et al.*[36] (electronarcosis), Barrera *et al.*[5], Alpers & Hughes[3] and Heilbrunn & Weil[43], all of whom found, on the whole, an absence of nerve cell changes and, on the other hand, Cerletti & Bini[17], Neubuerger *et al.*[76], Lidbeck[63], Ferraro *et al.*[29] and Ferraro & Roizin[30], who all observed definite changes in the nerve cells. I have been unable to find a satisfactory explanation of the lack of conformity in the observations of the respective authors on the grounds of the differences in their methods in this respect. This has also been the experience of earlier critics.

The choice of *method for sacrificing* the animals has also failed as a ground for reconciling the discrepancies. In principle, electrocution is presumably an unsuitable technique for the purpose of studying the possible neuropathological changes in the brain caused by earlier application of electric current. Cerletti & Bini[17] nevertheless applied a lethal current anobucally, whereas Globus *et al.*[36] applied it to the head. In the latter case this was of no importance, since the authors observed no neuropathological changes after electronarcosis. They also sacrificed some animals by ether, a method also used by Ferraro *et al.*[29] and Ferraro & Roizin[30] (with positive findings). Bleeding under anesthesia and similar techniques were used by Alpers & Hughes[3], Neubuerger *et al.*[76] and Winkelman & Moore[112], whereas Lidbeck[63] shot his animals through the heart and Barrera *et al.*[5] sacrificed their monkeys by air embolism.

The manner of sacrificing the animals is also of prime importance in view of the possible occurrence of post-mortem changes. Data regarding the lapse of time between death and fixation are, however, lacking in all the investigations, except in those in which perfusion fixation was used.

The *survival time* after the last ECT varies appreciably, from immediate death to 18 months. When nerve cell changes have been observed, an analysis of the literature reveals a certain tendency for such changes to have been more distinct when the survival time was short, *i.e.*, one or a few days only. This is particularly apparent with regard to pyknosis and in some reports, *e.g.*, those of Cerletti & Bini[17], Neubuerger *et al.*[76], Lidbeck[63], Ferraro *et al.*[29] and Ferraro & Roizin[30], to tigrolysis as well. However, the choice of survival time does not explain why other workers found no such changes.

A remarkable fact is the statement of presumably reversible changes observed even after several months' survival.

Finally, in order to assess the importance of *artefacts*, it is necessary to take into account the preparation of the histological specimens, fixation being possibly the most important factor. The majority of workers appear to have used formalin for immersion fixation. This does not exclude the possibility of appreciable variations, but the scanty data provided in most cases makes an analysis of the differences and similarities unfeasible. It is surprising that artefacts, such as shrinkage, caused by fixation are seldom discussed in this connexion. The question of staining methods will be dealt with in Chaps. 7 and 13.

The need of *control material* is all the more urgent. Without it, no worker who has used this technique is in a position to ascertain the extent of the artefacts—particularly with regard to fixation and post-mortem changes—or to check his own subjective scale of values.

Cerletti & Bini[17] had five dogs as controls; they considered this number to be on the low side. They expressed extremely critical views on the subject of comparisons and made reservations in view of the paucity of knowledge regarding the neuropathology of these animals.

Heilbrunn & Liebert[42], Alpers & Hughes[3], Fetterman[31], Heilbrunn & Weil[43], Neubuerger *et al.*[76] and Lidbeck[63] made no mention of control material. Barrera *et al.*[5] mentioned control animals but did not state their number or any other details; this also applies to Winkelman & Moore[112], Alexander & Löwenbach[1], Ferraro *et al.*[29] and Ferraro & Roizin[30]. Globus *et al.*[36] used one of their dogs as a control but sacrificed it by electrocution. Two cats served as controls in the investigation of Masserman & Jacques[68] and one monkey in that of Siekert *et al.*[93]

A further discussion of this question is found in Chapter 14 of the present study.

It will be evident from my experiments that the *age of the experimental animal* plays a decisive rôle in the occurrence of neuropathological phenomena in the brain. I made a special study of the literature in view of this matter and found that none of the writers already mentioned gave any data regarding the age of the experimental animals.

Earlier writers on the subject have focused little or no interest on *other pathological phenomena*, such as changes of the inflammatory type. In my opinion, the proliferation of the mononuclear cell elements in the immediate vicinity of the blood vessels reported in a few cases are suspect of encephalitis. LORENTZEN[65], in his study of the central nervous system in insulin shock, emphasized the relatively common occurrence of slight encephalitic changes in the brain of the rabbit. It is suggested that this fact should be borne in mind in all experimental studies on animals.

In the present investigation, use has been made of a procedure which I have denoted as an "unbiased examination". Masserman & Jacques[68] used a

procedure which was, in principle, similar. They submitted the brain of six cats subjected to ECT and of two controls to examination by different pathologists, who were unaware of whether the brains derived from shocked or from control animals. Obviously, no statistical analysis could be made of such limited material. With the exception of the aforementioned authors, no earlier workers appear to have made such an "unbiased examination". This procedure combined with a statistical analysis has presumably not been applied earlier in neuropathological studies on ECT.

C. SOME PHYSIOLOGICAL FACTS OF IMPORTANCE IN THE PRESENT STUDY

1. *Distribution of the Current in the Brain*

SMITT & WEGENER[95] found, on examination of human autopsy material, that only approximately 5—10 per cent of the potential drop between electrodes applied bitemporally to the intact head is received by the cerebral parenchyma, whereas 90—95 per cent is received by the integument. They found that the brain acted as a fairly homogeneous conductor and that the distribution of the current was somewhat diffuse, particularly if the electrodes were placed not too far anteriorly. The authors used a strength of current of 100 volts for 0.5 seconds.

Approximately the same results were obtained by HAYES[41] in experiments on a live monkey with intracerebral measuring electrodes. When the shock electrodes were applied bitemporally to the intact head, the distribution of the current was relatively diffuse and the maximum current density only exceeded the minimum density by about 30 per cent. The skull showed the greatest resistance. The resistance of the brain was found to be only about 3.5 per cent of the resistance of the intact head. This implies that the current is shunted over the skull before it is dissipated within the brain. Therefore, the size and placing of the electrodes presumably have no major influence on the distribution of the current in the brain in ECT. Hayes used a lower shock current, *i.e.*, 54 volts (58 m.a.) a dose comparable with that in clinical use.

In direct measurements on man with the actual shock current, JANSSON & SILFVERSKIÖLD[50a] found a resistance of 130—250 ohms between the electrodes.

Alexander & Löwenbach[1] made experiments on 23 cats. Nineteen of them received a single shock with large doses of current (60—2000 m.a., 120—520 volts, duration 2—10 seconds) and two animals repeated shocks at close intervals (6 and 52 shocks, respectively, 1400—1500 m.a., 550 volts, duration 0.40—0.60 seconds). The survival time varied between 4 minutes and 9 days. The interest of the authors was focused mainly on the vascular changes. In the animals subjected to large doses (500—1800 m.a.) in a single shock, they observed considerable vasoconstriction persisting from 5 minutes to one and

a half hours after the application of the current. With lower shock doses (300 m.a.) a brief period of arteriolar constriction was produced; it was noticeable 4 minutes but not 30 minutes after the shock. In both categories the vascular reaction was confined to the path of the current. The vasoconstriction was more pronounced with still higher doses, but lower doses—comparable with those used clinically—resulted in no such reaction. The authors concluded this fact to afford proof that the current is not distributed over the whole brain, but that it passes through a relatively limited path between the electrodes.

It is immediately evident that, for the purpose of establishing the path of the current, Alexander & Löwenbach's[1] data are not comparable with those of the other two workers cited. It is also possible that a stronger current flowing for a longer time is not distributed through the brain in the same way as the weaker and briefer current used in ECT. In other words, the inner resistance of the brain may undergo a change under the influence of the current. Alexander & Löwenbach observed this to occur in their experiments.

With regard to the more exact distribution of the current in the cerebral tissues, it may be presumed that the fluid in the ventricles offers less resistance and that possibly the blood vessels also act as better conductors than the cerebral parenchyma as a whole.

Broadly speaking, the existing experimental data warrant the conclusion that, with the doses of current applied in ECT, the current is distributed relatively evenly over the whole brain, with a moderate increase in the direct path between the electrodes. In other words, the brain behaves as a relatively homogeneous conductor.

2. *Effect of the Current on the Cerebral Blood Vessels*

ECHLIN[24] demonstrated that, in direct electric stimulation of the pial vessels in cats, considerable vasoconstriction resulted even from relatively low doses. Their strength was stated to be slightly less than the threshold dose for eliciting a motoric response in stimulation of the exposed cerebral cortex. The response was less marked in the pial vessels of dogs and monkeys. When, in the cat, the electrode was placed between the upper lip and one temporal muscle and a dose of 110 volts applied for 1—3 seconds, considerable constriction of the pial vessels was observed immediately after the current had been switched on.

Alexander & Löwenbach[1], whose investigation has already been discussed in the aforegoing, used larger shock doses. They also observed vasoconstriction of varying duration, mainly in the area between the electrodes.

SCOLZ & JÖTTEN[92] subjected five cats to a series of 10 electric shocks in close succession. The animals were sacrificed by decapitation. The survival time was in one case less than 5 minutes and in the others 5, 10, 20 and 40 minutes, respectively. The shock dose was 100 volts, corresponding to 200—250 m.a., with a duration of 0.5 seconds. Benzidine staining showed the blood vessels to

be constricted; there was thus cerebral anæmia with a spotty distribution over the whole brain. A tendency to a return to a normal degree of filling of the vessels was observed after 20 minutes and still more after 40 minutes. At its peak, the anæmia was approximately equal in all parts of the brain. A normal distribution of the blood throughout the brain was noted in one control animal. The survival time was short; it therefore permitted no definite conclusions regarding the prognosis of the slight changes found in the nerve cells in the brain of the treated animals.

Scholz[91] stressed that, in ECT, the seizure can scarcely be attributed to the cerebral anæmia. The onset of the seizure occurs immediately after the shock and it must therefore be a direct neuronal response to the electric stimulation. He considered that the cerebral anæmia is due to the actual seizure and not directly to the electric stimulation alone.

PART TWO

THE PRESENT INVESTIGATION: EXPERIMENTAL

CHAPTER 3

Statistical Methods

The results of the investigation, which are based throughout on comparisons between control and treated material, are mainly presented in the form of fourfold contingency tables. The statistical analyses of the tables were performed either with an exact two-sided method, in which case this is stated in brackets (exact method) or with Fischer's χ^2 method, corrected for continuity (denoted as χ^2).

MAINLAND[67] has set up convenient tables, calculated with an exact but one-sided method, showing the degree of probability for differences between two series of equal or unequal size, in the latter case with samples up to 20 in number. They are therefore particularly suitable for such relatively small series of enumeration data as those in the present investigation. It must, however, be pointed out that Mainland's tables are calculated only on one tail of the respective distributions. He gives the figures for the significance levels 0.025 and 0.005. Because two-sided comparisons were used in the other analyses in this investigation, Mainland's figures (given in brackets) have been multiplied by 2, and the significance levels 0.05 and 0.01 given instead of the corresponding figures 0.025 and 0.005 in a one-sided comparison. This obviously implies an approximation, but the risk of thereby being misled with regard to the probability is patently inappreciable. This risk is still further minimized by the fact that the results of the present investigation show that the control material did not, in any instance, exhibit greater changes than the treated material. It is therefore justified to assume that the conditions for the control animals do not involve any factor that might give reason to expect the comparison to reveal the reverse. As, however, it is customary to calculate the probability by means of a two-sided test, I adhered to this principle.

An account is given on page 49 of a special test for a particular estimation. This test is, even from a mathematical point of view, one-sided.

In those cases in which the results consisted of mean values for various

observations evaluated according to a scale with several grades, Fischer's t method was used for the comparison between the mean values for the respective groups.

The following degrees of significance are used:

$p < 0.001$ (highly significant)
$0.001 < p < 0.01$ (significant)
$0.01 < p < 0.05$ (almost significant)

As, however, I consider the numerical expressions to be more informative than the verbal expressions, the former are given in every case.

CHAPTER 4

The Material

a. EXPERIMENTAL ANIMALS

The experimental animals comprised a total of 57 cats: 41 were subjected to ECT's and 16 served as controls (see Table 2).

Table 2

The Material C = *Controls*

Cat No.	Specimen Nos.	Age Code No.	Weight grams	Anesth. Time Min.	Op. Time Min.	Living or Dead
9	94—105	3	3800	20	15	l
11	111—121	2	1360	20	10	d
13	140—151	3	3710	30	20	l
20	191—201	2	2600	40	20	l
43	501—517	3	3500	25	20	l
44	552—564	4	3080	25	15	l
45	525—538	2	2410	23	15	l
46	539—551	2	2040	20	12	l
47	565—577	2	2070	15	10	l
48	578—590	2	2950	16	10	l
49	591—603	2	2020	20	10	l
50	604—616	3	2920	20	10	l
51	617—629	3	2380	20	12	l
52	630—642	3	4460	25	18	l
53	643—655	2	2680	27	15	l
54	656—668	4	3800	25	15	l

The hygienic conditions were the best possible: the animals were kept in large dog-cages in which they could move about freely. Their diet consisted of fresh milk, fresh fish (Baltic herring) and, to a lesser extent, fresh meat.

Table 2 (Continued)

A = 4 ECT's*

Cat No.	Specimen Nos.	Age Code No.	Weight grams	Anesth. Time Min.	Op. Time Min.	Living or Dead	Survival Time
26	236—246	2	3360	30	18	l	24 hours
32	320—332	2	3400			l	»
33	306—319	3	3750			l	»
55	669—681	2	2810	15	12	l	»
56	682—695	5	3330	18	12	l	»
57	696—708	2	2160	20	13	l	»
58	709—721	2	3200	19	13	d	»
59	722—734	4	3800	20	13	l	»
60	735—749	4	4000			l	»
34	376—388	2	3500			d	48 hours
35	362—375	2	2650			l	»
65	804—817	2	3000	18	13	d	»
66	818—831	3	3400	17	12	l	»
67	832—845	5	3220	23	15	d	»
68	846—859	1	1550	16	10	l	»
36	389—406	2	3000			l	96 hours
37	407—419	3	2930			l	»
38	420—436	3	2620	26	20	l	8 days
39	437—451	3	2320	25	15	l	»
61	750—763	3	4800	20	16	l	»
62	764—777	3	3000	20	15	l	»
63	778—790	3	3050	20	15	l	»
64	791—803	2	3400	21	12	l	»

* 4 ECT's at 2-hour intervals.

This appears to rule out the risk of any vitamin deficiency. All the animals lived under identical conditions during the entire experimental period.

All the animals were, as far as it was possible to ascertain, healthy at the beginning of treatment* or, in the case of the controls, immediately before they were sacrificed. The appetite was good in every instance; this also applied to the shocked animals during the course of treatment.

The weight of the respective animals was recorded.

* One animal was excluded from the series because it showed signs of ill-health; it died shortly afterwards. Diarrhoea developed in two animals (nos. 14 and 15) after the ECT's had been started; since they showed no other signs of illness they were included in the series.

Table 2 (Continued)

B = 11—16 ECT's*

Cat No.	Specimen Nos.	Age Code No.	Weight grams	Anesth. Time Min.	Op. Time Min.	Living or Dead	Survival Time
28	261—275	2	2800	40		l	48 hours
29	276—290	2	2600	20		l	»
27	291—304	4	4500	30		l	96 hours
30	349—361	4	4670	34		d	»
31	333—348	3	3500	43		l	»
40	452 a—467	2	1400	30		l	8 days
41	468—483	2	1830	25		l	»
42	484—500	2	2480	15		l	»

* 11 ECT's. Cats nos. 27, 28, 29.
1st day: 4 ECT's at 2-hour intervals.
2nd day: do.
3rd day: 3 ECT's at 2-hour intervals.
16 ECT's. Cats nos. 30, 31, 40, 41, 42.
1st—4th days: 4 ECT's at 2-hour intervals.

Table 2 (Continued)

D *and* E = *Various* ECT's

Cat No.	Specimen Nos.	Age Code No.	Weight grams	Anesth. Time Min.	Op. Time Min.	Living or Dead	No. of ECT's	Frequency	Survival Time
22	224—235	2	2670	30	23	l	4	1/day	24 hours
23	247—257	2	2830	27	18	l	»	»	»
5	71—82		3460	35	20		10	3/week	90 hours
12	122—134	2	1860	20	14	d	7	3/week	92 hours
19	213—223		2350			d	11	3/week	5 days
21	202—212	2	1950			l	12	3/week	2 days
17	176—187		2730			l	9	3/week	10 days
15	164—175		4450			l	9	3/week	10 days
14	152—163		3625	28	20	d	9	3/week	10 days
10	83—93		2460	20	10	l	2	1/day	18 hours

The animal experiments were carried out over a period of almost two years. For this reason it was not always feasible to make a random selection, but the animals were assigned to the various groups as they were delivered and as the need arose. They were, however, as far as possible distributed at random

among the various groups. It is apparent from the distribution of the age and body weight (pp. 30 and 31) that there was no appreciable difference between the composition of the respective groups. This also applies to the sex distribution; it was therefore considered warranted to omit this factor from the analyses.

b. GROUPING

Earlier workers have, as a rule, used the same frequency as in clinical ECT, *i.e.*, 2—3 shocks weekly. The animals were allowed to survive for a relatively long or short period—mainly the latter. The object was obviously to imitate as closely as possible the clinical course, in order to obtain results in animals that would provide information regarding the conditions in ECT for therapeutic purposes in man. The drawback is evident, namely a considerable variation in the survival time after the individual shocks. This might result in a very heterogeneous conception of possible sequelæ of the treatment. This applies in particular if—as is probable—such sequelæ undergo changes with time, if they are not perceptible with histological techniques until a certain period has elapsed and if they are reversible within a certain time. These considerations led me to abandon the so-called clinical treatment used in the beginning of the investigation; the animals were instead treated according to the following system.

Group C: controls.

Group A: 4 ECT's at 2-hourly intervals, survival time (ST) after the last shock 24 hours, 48 hours, 96 hours or 8 days, respectively; the sub-groups were therefore denoted as A_{24}, A_{48}, A_{96} or A_{8d}.

Group B: 11—16 ECT's, 4 daily, on 3—4 consecutive days. The shocks were given at approximately the same time each day. The interval between the shocks was 2 hours and between each series of 4 shocks 16 hours. The survival time after the last shock was 48 hours, 96 hours or 8 days, respectively. The sub-groups were therefore denoted as B_{48}, B_{96} or B_{8d}.

Group D: 1 ECT daily on 4 consecutive days, the survival time after the last shock being 24 hours.

Group E: remaining animals, given ECT's with the clinical frequency.

c. AGE DISTRIBUTION

Unfortunately, the exact age of the animals was unknown. It was, however, possible to classify them to some extent according to age on the basis of their general appearance and of the observations made on the operating table, especially of the degree of ossification and the fragility of the bones and—in some degree—according to the body weight.

A five-grade scale was used for the first classification; the results are shown

in Fig. 1, from which it may be inferred that the whole material was relatively homogeneous and normally distributed in this respect. Such a fine gradation must, however, be subject to considerable uncertainty. In the subsequent calculations, the material was therefore divided into two groups only: one of younger animals (comprising groups 1—2) and the other of older animals (comprising groups 3—5). Obviously, there are a few animals in the borderline zone between the two groups of which the classification is uncertain. On broad

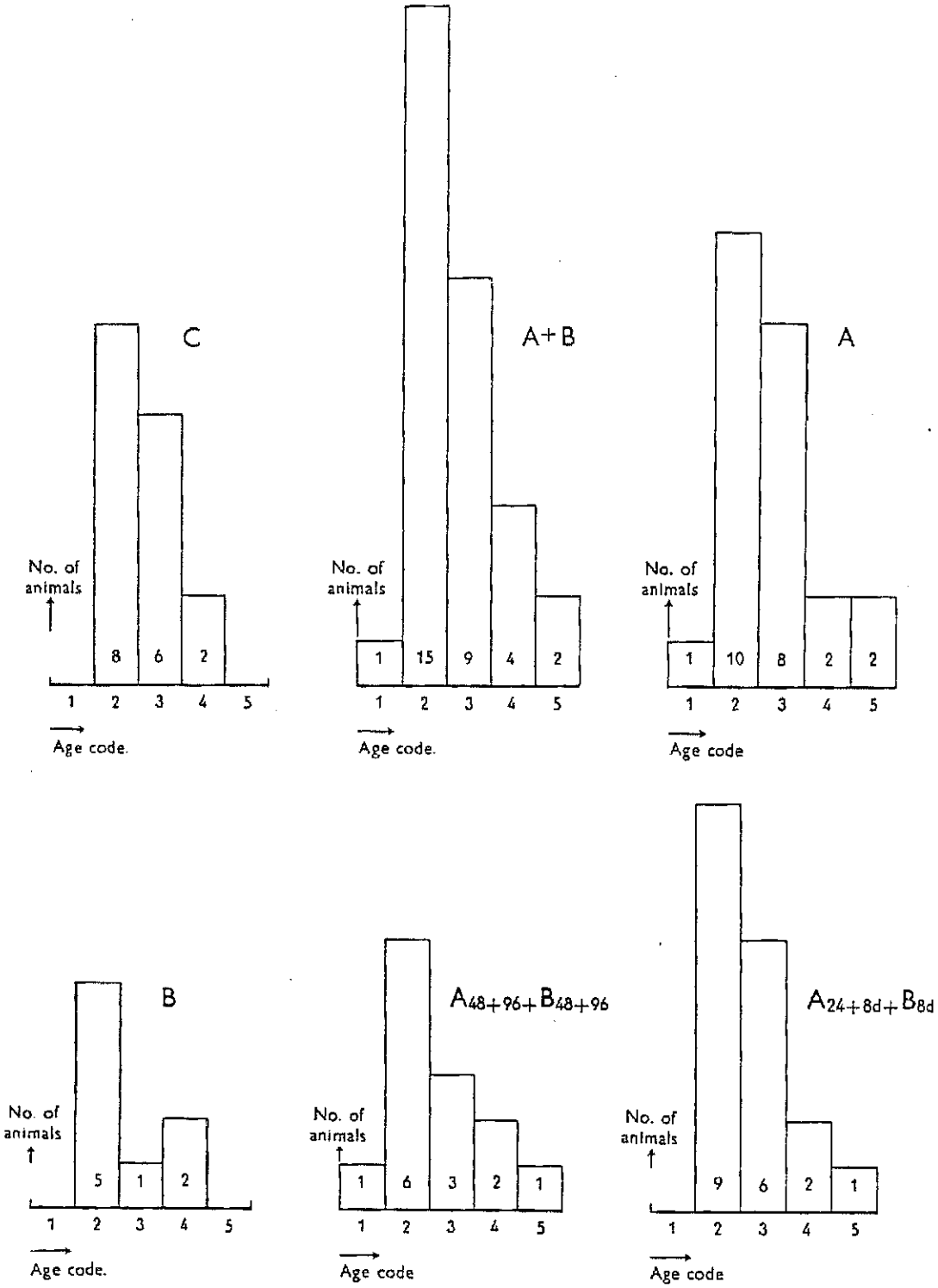

Figure 1. Age distribution in the different groups. C = control animals, A = animals with 4 ECT's, B = animals with 11—16 ECT's.

lines, however, such a classification into two groups only should be relatively reliable. Neither very young cats nor those obviously advanced in age were included in the material.

The following schema shows that the proportion of younger and older animals was approximately the same in the respective categories, *i.e.*, controls and animals subjected to ECT.

C	$\frac{\text{Younger animals}}{\text{Older animals}} \cong \frac{6}{6}$	A do.	$\cong \frac{11}{12}$
B	do. $\cong \frac{5}{3}$	A + B do.	$\cong \frac{16}{15}$
$A_{48+96} + B_{48+96}$ . . .	do. $\cong \frac{7}{6}$		

d. DISTRIBUTION OF THE BODY WEIGHT

Each animal was weighed immediately before it was sacrificed; this weight was recorded as representative for the animal in question. The mean figures were as follows. Group C: 2.86 kg; Group A: 3.14 kg; Group B: 2.97 kg; Groups A + B: 3.10 kg; sub-groups $A_{48} + A_{96} + B_{48} + B_{96}$: 3.18 kg and sub-groups $A_{24} + A_{8d} + B_{8d}$: 3.04 kg.

As was the case for the age distribution, no significant difference was found with regard to the distribution of the body weight in the respective groups.

CHAPTER 5

The Shock Treatment

a. TECHNIQUE FOR THE ELECTRIC SHOCKS

The shocks were given with the McPHAIL-STRAUSS[104] plexacon apparatus which, with a condensor discharge of a known quantity of electricity measured in joules (J), delivers a rapidly attenuated monophasic or diphasic oscillation of the electric current through the head of the animal.

The electrodes were of silver; they were round and the diameter was 15 mm. In every case they were applied symmetrically immediately in front of each ear (bitemporally and frontally). The hair in this area was shaved off, the scalp moistened with saline solution and electrode jelly rubbed in. The position of the electrodes was such that a line drawn through their respective centres passed through the frontal part of the brain (see Fig. 2).

During the seizure, the animal—and in particular its head—was protected manually from striking against the table on which it lay.

The quantity of current was kept as low as possible, *i.e.*, monophasic current $\bar{x} = 2.2$ joules (range 0.6—6.0 joules, $n = 246$); diphasic current $\bar{x} = 3.6$

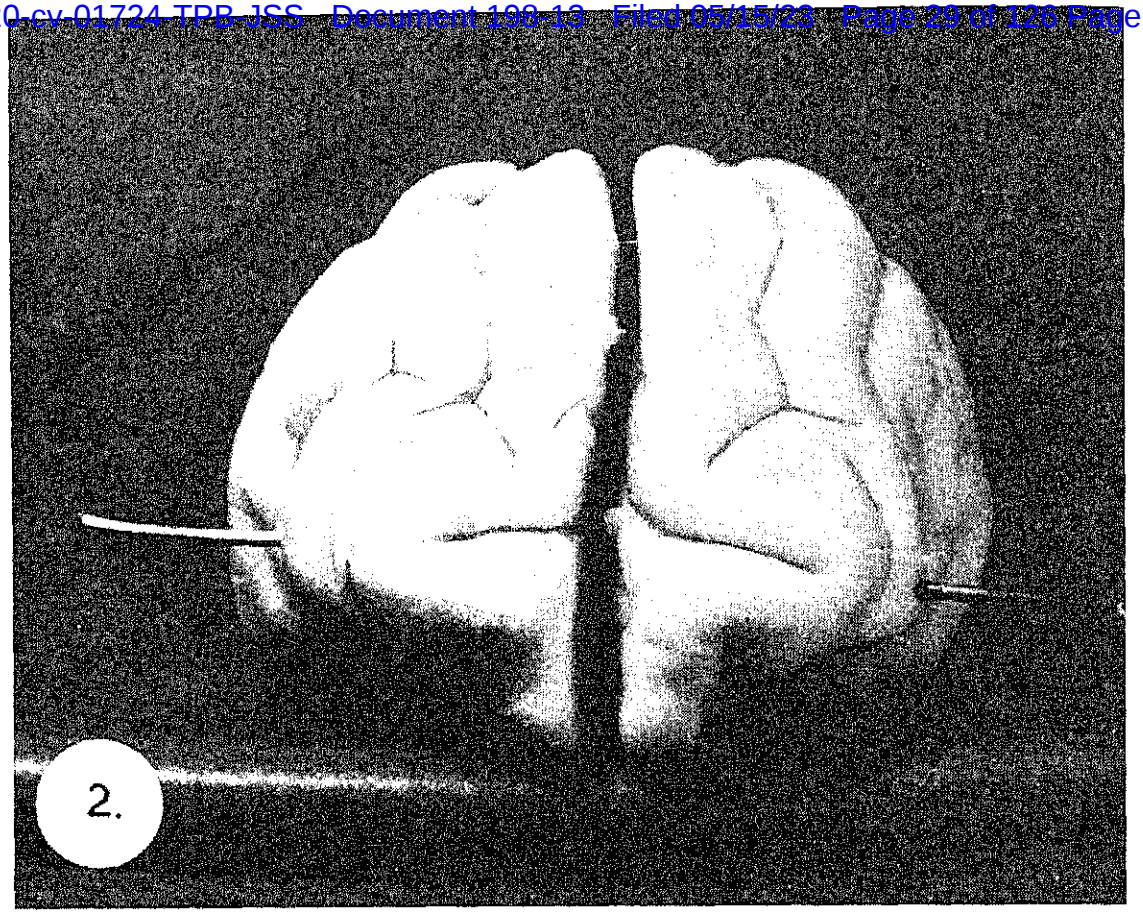

Figure 2. A cat's brain, frontal view. The probe shows a line drawn through the centres of the electrodes. The length of the probe is 53 mm.

joules (range 2.0—6.0 joules, $n = 54$). One cat (no. 5) was, however, subjected to considerably larger doses (from 12 joules, monophasic current, and downwards); this was in the first experiments with the apparatus.

b. THE SEIZURES

The reaction of the cat to shock-producing electric current strongly resembles the reaction of human subjects. Thus, if the strength of current is sufficient, typical grand mal epileptic seizures occur, it being possible to distinguish the various stages, *i.e.*, latency time and the tonic and clonic phases. If the dose of current is insufficient, abortive seizures occur. The convulsions seldom have an atypical course.

Altogether 280 shocks were given to the 41 cats in the series; 265 (94.6 per cent) were typical and 15 (5.4 per cent) atypical. The abortive seizures amounted to 33; they were not included in the total.

The typical seizure was ushered in by a *latency time* of a few seconds (see Fig. 4). By this is meant the period elapsing between the time the current was switched on—the animal, immediately after an initial twitch at the rush of current, collapsing with all its muscles relaxed—and the onset of the muscular

spasm. This started with a tonic flexor spasm, particularly marked in the fore legs. The forepaws were brought up over the head in slow motion, passed in front of the eyes and then became fixed in an intense extensor spasm—the paws pointing downwards and backwards—during the entire convulsion. This

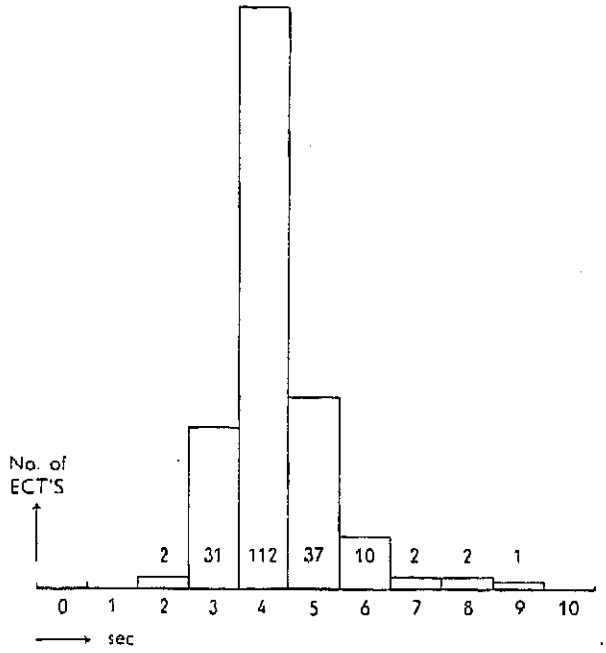

Figure 4. Latency time. $n = 197$ seizures.

extensor spasm was still more marked in the hind legs, the muscles being so strongly contracted that they were as hard as a board. The back was moderately arched. The whole appearance of the animal was that of being arrested in the middle of a powerful jump. If it was lifted, it felt like a board.

The interval between the onset of the flexor spasm and the first clonic twitches has been denoted as the *tonic phase* (see Fig. 5). The clonic twitches usually had a gradual onset, increased in intensity and reached a maximum, after which they decreased in intensity and frequency. They were not infrequently terminated by more co-ordinated running movements. These running movements occurred in 2 out of the 33 abortive seizures, 4 out of 14 atypical and in 147 out of 220 typical seizures ($100 \times 147/220 = 66.8$ per

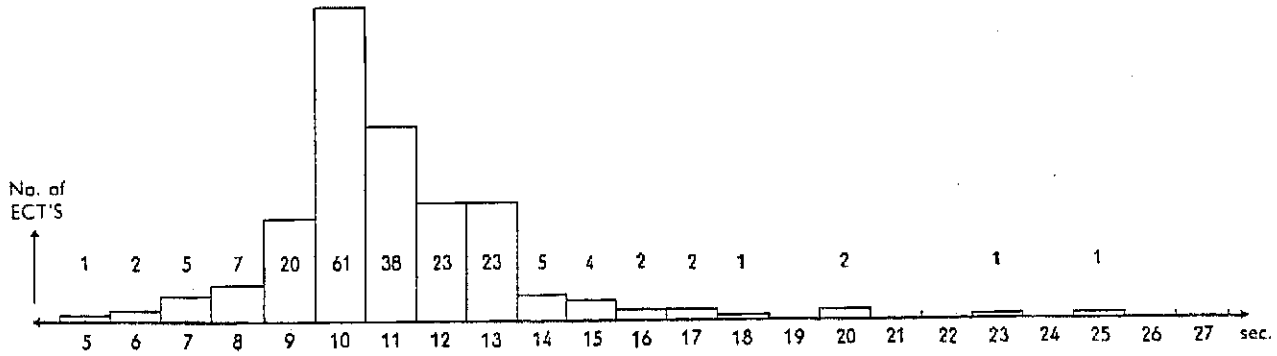

Figure 5. Tonic phase. $n = 198$ seizures.

cent)*. Although the onset of the *clonic phase* (see Fig. 6) was well marked, it was more difficult to distinguish its termination. The running movements were not, however, included in the duration of the clonic phase.

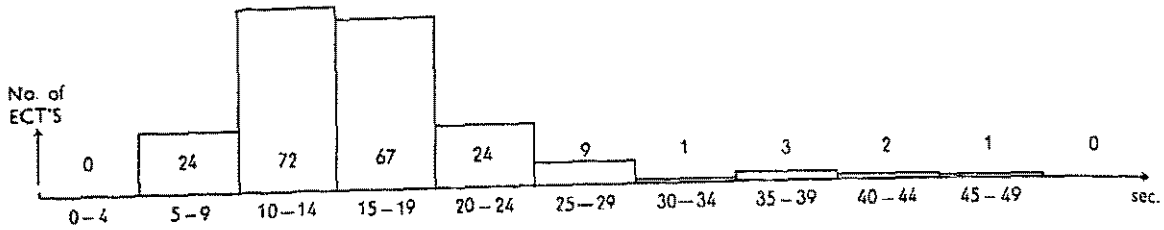

Figure 6. Clonic phase. $n = 203$ seizures.

The subsequent stage, lasting until the animal had recovered sufficiently to exhibit spontaneous movements—usually standing up or starting to react to sensory stimuli by turning its head in the direction of a sound or a visual impression—has been denoted as the *coma stage* (see Fig. 7). It was obviously still more difficult to determine the duration of this stage.

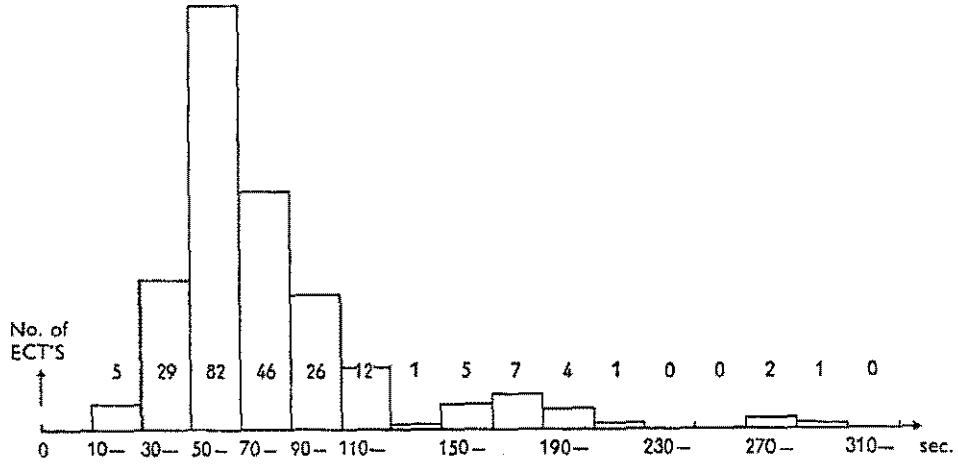

Figure 7. Coma stage. $n = 221$ seizures.

After the coma stage, the animal usually exhibited moderate blunting of the sensory functions and poorly co-ordinated movements—marked ataxia, more evident in the hind legs than in the forelegs. Ataxia was present in all the animals immediately after the typical seizures. Transitory hyperirritability with strong startle reactions occurred fairly often, but generalized lassitude was more common. When hyperirritability was present, it subsided within a few minutes and was succeeded by lassitude. It occurred after 53 out of the 220 typical and 2 out of the 33 abortive seizures.**

* No record was made of this phenomenon in the early stages of the investigation.

** One cat (no. 62) reacted immediately to the electric current with a violent attack of rage; there was impaired mental acuity but not complete unconsciousness (sham rage). This occurred on the first shock (shock dose 2 J, monophasic current) and was accompanied by involuntary urination.

Immediately after the seizure was over, the animal was sluggish but not timid. It often curled up in a corner of the cage but did not withdraw if it was touched. If it was dropped from a height, it curled up at once after landing. This sluggishness decreased successively, but lasted for several hours in some cases. After repeated shocks (four daily) it could even be observed 24 hours later—although to a less marked extent—in a few cases.

Tachypnœa lasting for a few minutes occurred directly after the seizure in a few instances. No tachypnœa was observed after the 33 abortive seizures or the 14 atypical convulsions. In the 220 typical seizures it was pronounced in 8 cases and very slight in 9.

Phenomena of autonomic origin, such as increased salivation, horripilation, urination and defæcation also occurred. The two first-mentioned were practically regular features, whereas the last two were less frequent.

When the ECT was repeated, the animals showed no fear of the apparatus or of the application of the electrodes, provided that the earlier seizures had been of the grand mal type. This indicates complete amnesia for the convulsion. This did not appear to be the case when abortive seizures were concerned; the animals then exhibited fear of the apparatus.

It is thus evident from the aforegoing that, on the whole, the reactions of the cat to ECT are very similar to those of man.

c. RELATIONSHIP OF THE SHOCK DOSE TO THE AGE

As pointed out earlier, the effect of an insufficient dose of current was an abortive seizure only. This afforded the possibility of determining the minimum quantity of electricity required to produce a generalized seizure in the individual animals.

This dose, the *threshold dose*, appeared to vary in the different animals but to remain relatively constant in the individual case. It was, however, possible to discern a slight tendency to an increase in the course of treatment. Thus, the threshold dose might be slightly higher at the end of a series of ECT's than at the beginning. I did not make such determinations systematically. This was because an abortive seizure was found to cause uneasiness, as opposed to the generalized seizure, which was accompanied by complete amnesia for the attack. Nevertheless, my endeavours to keep the shock dose as low as possible resulted in a number of abortive seizures sufficient to form a series which could be subjected to a statistical analysis. This indicated that the threshold dose was lower for the younger animals than for the older ones.

When a comparison was made between the animals subjected to ECT* in

* The first 5 animals were not included, since the method was not standardized in this respect at the beginning of the experiments.

the younger group and those in the older group, it was found that the threshold dose for 14 out of the 18 cats in the younger group was definitely ≤ 2 joules monophasic current, the threshold dose in no case being definitely over 2 joules. Four cats in this group had no abortive convulsions; it was therefore impossible to determine their threshold dose exactly (*cf. infra*).

In the older group, 4 out of the 14 cats had a definite threshold dose of ≤ 2 joules monophasic current and 7 cats definitely over 2 joules. Abortive seizures were lacking in 2 of the animals.

In the older group a diphasic value was converted into a monophasic value by multiplying the former by the factor $\frac{5}{4}$. This did not change the distribution of the animals with regard to the threshold dose.

The following schema shows the distribution of the animals according to the shock dose and the age:

	No. of younger animals	No. of older animals
Threshold dose ≤ 2 J m.	14	4
» » > 2 J m.	0	7

Difference: $\frac{14}{0} \sim \frac{4}{7}$

$p < 0.001$ (exact method: 0.0007)

It may be objected that the missing values might affect this difference. It was, however, possible to estimate the limits within which the missing threshold doses lay. They were as follows: in the younger group $1-2.25$, < 2.5, < 3 and < 5 joules monophasic current, and in the older group < 5 and < 5, respectively. The risk therefore appears to be inappreciable.*

Pending more conclusive data, it is only permissible to draw the following inference. There is a definite tendency for younger animals to require a lower dose of electric current than older ones to produce generalized grand mal seizures.

CHAPTER 6

Preparation of the Specimens

a. ANESTHESIA AND REMOVAL OF THE BRAIN

At the appointed time after the last ECT, the animal was anesthetized, usually with ether, and the cerebrum removed by means of a simple operation. The procedure was the same for the control animals; all the animals were therefore numbered in the order in which they were operated on (see Table 2). The

* One animal belonging to the older group (Cat no. 60) had atypical seizures only; shock dose 3–5 J. In one experiment (Cat no. 37) a lead was faulty; the value is therefore somewhat uncertain.

animal was then alive in the majority of cases. The cerebellum was removed at a later session; this procedure was necessitated by the fact that the tentorium cerebelli of the cat is ossified. The interval elapsing between the induction of anesthesia and the removal of the brain is denoted as the *anesthesia time*. The mean figure for its duration was 23.9 minutes (22.9 minutes in the controls and 24.5 minutes in the cats subjected to ECT). Cats 19, 20, 21, 22, 23 and 26 were given pentothal sodium by the intraperitoneal route with a little ether as an adjuvant.

The mean duration of the *operation time*, *i.e.*, the time from the beginning of the operation to the final removal of the cerebrum, was 8.0 minutes less than the anesthesia time.

The large majority (49 cats) were alive when the cerebrum was removed, but 8 animals (1 control and 7 subjected to ECT) died shortly before. Death may presumably be attributed to the loss of blood during the operation (the calvaria of the cat is extremely well vasculated) or possibly to some extent to asphyxia —*cf.* Table 2.

b. CHOICE OF THE SPECIMENS

Immediately after removal of the cerebrum, blocks a few millimetres in size were cut with a sharp pair of scissors from the following areas (see Fig. 3):

gyrus sigmoideus anterior dexter et sinister*
corresponding to the area frontalis agranularis (6) and area frontalis granularis (8)
gyrus sigmoideus posterior dexter et sinister
corresponding to the area præcentralis (4)
gyrus lateralis dexter et sinister
corresponding to the area præparietalis (5)
gyrus ectosylvius posterior dexter et sinister
corresponding to the area temporalis media (21)
cornu Ammonis dexter et sinister

Specimens of the cerebellum (parts of the lobulus posterior and lobulus ansatus) were subsequently taken in the same way.

The technique was uniform for all the brains and the topography was as exact as possible.

This preparation required only a few minutes. The specimens were fixed in Carnoy's solution (glacial acetic acid, chloroform and absolute alcohol, 1: 3: 6) freshly prepared on each occasion. After remaining for a sufficient time in Carnoy's solution and then in absolute alcohol, the specimens were embedded in paraffin and cut in sections 5 μ thick.

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* According to WINKLER & POTTER[113], who used the nomenclature of Flatau & Jacobson.

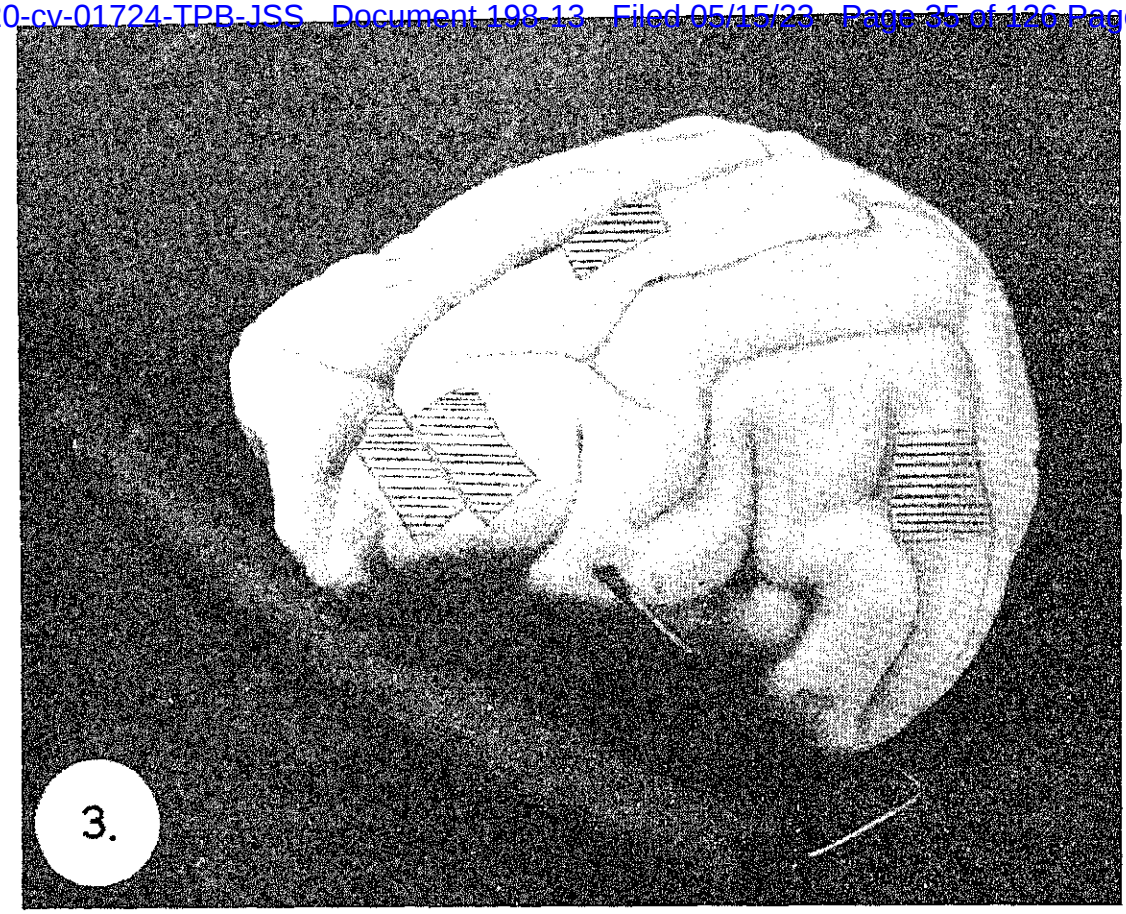

Figure 3. A cat's brain, oblique frontal view. The parts of the cerebral cortex examined are hatched. From left to right: gyrus sigmoideus anterior, gyrus sigmoideus posterior, gyrus lateralis, gyrus ectosylvius posterior.

After a number of experiments with toluidine blue and hæmatoxylin and eosin, these staining methods were abandoned. All the specimens were subsequently stained with Einarson's gallocyanin-chromalum method, using his instructions.

CHAPTER 7

Staining Method

Reference is made to EINARSON's publications[25, 26] for a complete account of the theoretical aspects of the gallocyanin-chromalum staining method. Since the Nissl substance in the cytoplasm of the nerve cells is strongly basophilic, these cells are particularly suitable for staining with this technique. Einarson stressed that the method is "exquisitely progressive"; the staining does not, therefore, become more intense if the specimen remains in the staining solution longer than is necessary for "maximal occupation by the stain of the nucleoproteins". He also made the following statements. "Owing to the great stability of the staining it is also completely unaffected by alcohol

xylol during the procedure of dehydration and mounting". Thus, "the staining intensity depends directly on the inherent capacity to bind the stain, possessed by the living cell at the moment of fixation".

There is, however, an unspecific binding of the dye—not dependent on the basophilia—but it may be reduced to negligibility by keeping the pH sufficiently on the acid side. A suitable pH is 1.64 (1.50—1.75). The unspecific co-staining is namely due partly to a purely physical adsorption, which is a function of the pH of the staining solution, and partly to a binding to the proteins of the tigroid substance. The latter will not occur on the acid side of the isoelectric point of the proteins (pH 2.7). In the case of nervous tissue, the unspecific adsorption is manifested in an increased stainability of the interstitial tissue—the background.

Einarson also cautioned against using dye solutions more than three to four weeks old.

He pointed out that "the staining consists in a selective binding of the lake cations to the phosphoric acid groups of the polynucleotides, *i.e.*, there is formed a blue salt of the lake cations and the nucleic acids of the cell structures".

The method has been used by Einarson and other workers—mainly in Denmark—particularly for studies of the nerve cells.

It has been possible to observe different degrees of stainability in the nucleus and the cytoplasm. Einarson, as cited by Lorentzen[65], distinguished between the following groups.

1. *Hyperchromatic or slightly and moderately chromophilic cells.* The cells were deeply stained and the Nissl substance was usually close to the nucleus. The nucleolus was intensely stained and a relatively large number of particles were present in the nucleus.

1 a. *Extremely chromophilic cells.* The cells were intensely stained, with closely packed Nissl granules in the cytoplasm, well defined cell boundaries and a strongly stained nucleus. The nucleolus was often visible, despite the dark colour of the nucleus, whereas the nuclear membrane was scarcely discernible. The dendrites were often stained for a considerable distance. The condition was stated to be reversible in many cases but could also progress into irreversible sclerosis.

2. *Chromophobic cells.* The cells exhibited a decrease in the number of Nissl granules and an increase in the nuclear chromatin. The nuclear membrane was intensely stained and the nucleolus enlarged. Perinuclear accumulations of the Nissl substance and nuclear caps were also present, but the Nissl substance was sometimes assembled in the periphery of the cytoplasm.

3. *Extremely chromophobic cells.* There was little or no chromatin. Both the nucleus and the cytoplasm were shadow-like and the nucleus was frequently eccentric. The picture thus resembled that of chromatolysis.

0. *Chromoneutral cells.* These cells constituted the normal types around which the others were grouped.

The different degrees of chromaffinity* were compared with the various stages of functional activity. Group 1 thus represented cells in initial activity. Group 2 was considered to show cells in an "increasing, strong, and prolonged state of activity" and group 3 cells "gradually approaching fatigue and exhaustion".

The aforementioned observations are, on the whole, in agreement with the quantitative data on the nucleic acids of the nerve cells obtained with a more exact method (CASPERSSON's[15, 16, 58] ultra-violet spectrophotometry) in particular the work done by HYDÉN *et al.*[47, 48, 44, 39]

It may be inferred from the present study that I was extremely cautious with regard to the more intensely stained cells—the "dark cells". This was on two grounds. Firstly, the "dark cells" may be artefacts, produced by a mechanical action on the unfixed material (see p. 79). Secondly, shrinkage caused by fixation may result in an increase in density. It is namely possible in certain cases to observe considerably dilated pericellular spaces surrounding these "dark cells". There is thus reason to suspect that the changes in volume caused by fixation may give rise to an erroneous interpretation. The changes in volume following fixation with the medium used in the present investigation—*i.e.*, Carnoy's solution—are presumably mainly, or exclusively, in the form of a decrease in volume, with resulting increased density.

It therefore follows that a decrease in stainability—chromophobia—is less subject to artefacts caused by fixation, since it cannot reasonably be ascribed to shrinkage. On the other hand, it may be envisaged as due to the extraction by the fixative of one or several cell components. It has been shown by Einarson that staining with gallocyanin-chromalum is, under certain conditions (among them a suitable pH), almost entirely caused by a binding of the dye to the nucleic acids in the cell structures (*vide supra*). Fixation with Carnoy's solution results in extremely good preservation of these cell components in particular, with practically no extraction.

The question of various kinds of artefacts will be discussed in a later section of this paper, on the basis of the experience in the present investigation. At present, I only wish to point out that the phenomenon of chromophobia appears to be fairly specific, whereas chromophilia, pyknosis, "dark cells" and shrinkage are more difficult to evaluate and to distinguish from each other. It seems to be relatively easy to differentiate between chromophobic cells of Einarson's type 2 and shrunken cells or SHARRER's[89] "dark cells", respectively. This will be discussed in more detail later.

Einarson and his co-workers have stressed that the gallocyanin-chromalum staining method affords a considerably more reliable basis than the earlier

* Here and in the following, the term chromaffinity denotes the ability to take up the dye.

regressive staining methods with basic aniline dyes for an estimation of the basophilia of the cytoplasm of the nerve cells. They expressed the opinion that the older methods are unfit for evaluating the activity changes of the nerve cells on the basis of their content of the Nissl substance. I found the gallocyanin-chromalum method to be superior to a regressive method (toluidine blue staining) for analyses of the present material. I therefore consistently followed Einarson's procedure in staining all the specimens in my material.

PART THREE

THE PRESENT INVESTIGATION: NEUROPATHOLOGICAL OBSERVATIONS

CHAPTER 8

Macroscopical Observations

Both on exposure and on removal of the cerebrum, its appearance was studied in view of possible pathological phenomena. After blocks had been cut for histological examination, the remainder was cut into sections and carefully examined, in some cases under a magnifying lens, for hæmorrhages and other gross changes. If signs of hæmorrhage were found, specimens were taken for histological examination in order to ascertain whether this was actually the case and, if possible, to establish the age of the hæmorrhage. The incidence of hæmorrhages will be dealt with later (p. 58).

In 8 cases the brain substance appeared to be œdematous; hæmorrhages were also present in 7 of these cases and lacking in 1. It must be stressed that this observation was macroscopical and subjective only. All the cats in question had been subjected to ECT. In one case (Cat no. 36) a small meningeal adhesion was visible under the site of the electrode and in another (Cat no. 40) the meninges appeared to be somewhat thickened. I do not attach any great importance to these findings. On the whole, no changes in the cerebrum other than the hæmorrhages were discernible macroscopically. In no instance did the cerebellum exhibit any macroscopical changes, hæmorrhages not excepted.

CHAPTER 9

Histological Evaluation: Method and Diagnostic Criteria

The material, comprising approximately 700 specimens, was examined three times.* At each of the three examinations, all the specimens taken from a

* A fourth examination, of which an account will be given in Chapter 15, was made of a limited part of the material, as an additional check up.

particular part of the brain were studied in sequence. For example, at the first examination, all the specimens of the gyrus sigmoideus anterior from all the animals—both controls and those subjected to ECT—were examined consecutively. All the specimens from another part of the brain—for example, the gyrus lateralis—were then examined.

At the following examinations, the procedure was repeated with certain modifications. Other sections, at least two from each specimen, freshly stained and mounted, were used. With the help of a mechanical stage, the entire surface of each section was examined systematically, visual field by visual field.

The microscope was a Leitz' binocular ortholux with the following optics: *objective:* 16 mm apochromate, numerical aperture 0.30; 8 mm apochromate, numerical aperture 0.65; 4 mm apochromate, numerical aperture 0.95 and 2 mm oil-immersion apochromate, numerical aperture 1.32; *ocular:* periplane 8 ×. With these combinations, magnifications of 120, 240, 400 and 900 × were obtained. The two lower magnifications were used systematically for the whole section, and the two higher as required. The same microscope was used throughout the investigation.

These procedures were time-consuming; thus, at the third examination, I could only deal with 10—12 specimens a day. Up to six weeks were therefore required for each part of the brain at this examination.

The question then arises of the purpose served by these examinations.

The *first examination* provided an orientation of the existing phenomena and served as a basis for drawing up a form for the protocols.

At the *second examination*, these forms were used for recording the observations made. My object was to find differences (or similarities) between the controls and the different groups of cats subjected to ECT and, as regards the latter category, between the various groups and sub-groups.

In this examination, as in the first, I was aware of the origin of the respective specimens, *i.e.*, whether they were taken from a control animal or from one subjected to ECT.

The second examination resulted in a number of recorded data, usually graded according to a particular scale. These data were assembled for statistical analyses, on the basis of which a comparison could be made between, for example, the controls and the animals treated with ECT. However, for critical reasons—mainly because the examination was not unbiased, a point which will be discussed later—I abandoned this procedure and the majority of the figures obtained will therefore be omitted from this report.

A *third examination* of the material thus became necessary. I then made use of the observations made in the second examination as follows. The most reliable findings—*i.e.*, those which, at the second examination, seemed to provide the best basis for distinguishing between the controls and the treated animals—were taken as diagnostic criteria at the third examination. They are

recorded in the following in the order in which the second examination they appeared suitable for permitting a differential diagnosis between the control animals and those subjected to ECT in an unbiased evaluation.

Diagnostic Criteria

I. *Changes in the vessel wall and its immediate vicinity:* dilated perivascular spaces of irregular shape, forming sacs, in which scattered histiocytic elements were sometimes present. Glial reactions in the immediate vicinity of the vessels were also included.

I A. Occasional *free pigment* in the aforementioned sacs or, more infrequently, phagocytozed pigment in the cytoplasm of the histiocytes.

II. *Glial reaction in general:* increase in the number of glia cells in the cerebral parenchyma and particularly an increase in the number of satellite cells surrounding the nerve cells—satellitosis.

II A. *Satellitosis* appeared to be a relatively specific phenomenon; it was therefore graded and recorded separately. It consisted of an increase in the number of satellite cells, which were often swollen and lay in indentations in the cytoplasm of the nerve cells.

Sporadic cases of true neuronophagia also belonged to the category of glial reactions.

III. *Changes in the nerve cells:* It appeared easier to record a greater variation in chromaffinity from nerve cell to nerve cell than to grade the chromaffinity in general of the cell population. A special phenomenon found in certain pyramidal cells, *i.e.*, nuclear hyperchromatism with coincident relative chromophobia in the cytoplasm, particularly in its periphery, also seemed to be of great value in substantiating the diagnosis "shocked". Particular note was made of focal accumulations of such cells and of a greater variability of nerve-cell chromaffinity in circumscribed areas.

Such nerve-cell phenomena as shrinkage, spike-like processes and corkscrew formations were also noted and graded, although no diagnostic importance was attached to them. Neither vacuolization* nor actual swelling was observed. When diagnosing a specimen as "shocked" or "not shocked", respectively —see p. 46—fresh terminal hæmorrhages were not taken into consideration, although they were recorded. (It was only after the examination was concluded that I found, in a routine calculation of the incidence of hæmorrhages in the two categories of specimens, the statistical difference mentioned later—(*cf.* p. 58).

The occurrence of small, scattered round-cell infiltrations in a number of animals—both controls and those that had undergone ECT—was recorded

* Except in the cerebellum.

but not included in the diagnostic criteria. This group could be distinguished from the aforementioned characteristic changes in the vessel wall and was found in both categories. I therefore considered that it could be disregarded in this connexion.

*

At the *third examination*, my aim was an unbiased examination. This was based on the fact that I did not know whether the specimen to be examined derived from a control animal or from one subjected to ECT. I did, however, know from what part of the brain the specimen was taken.

The procedure was the following. For reasons of economy, sections from the same part of the brain of one animal, taken from both hemispheres—*e.g.* from the left and right gyrus sigmoideus anterior—were placed on the same slide. The labels on all the slides with sections from the relevant part of the brain—both of control animals and those subjected to ECT—were covered and the slides thoroughly shuffled. Slides were then selected at random. A protocol, on which were noted all observations and their grading, was drawn up for each slide. The same form, slightly modified (see Appendix I) was used as that for the second examination. Finally, I made a diagnosis: shocked or not shocked, with the help of the diagnostic criteria enumerated earlier. Only when the diagnosis had been made was the cover removed from the label and the number recorded. The procedure was uniform for specimens from all parts of the brain.

At suitable intervals and particularly when the examinations had been interrupted—for example at a week-end—I re-examined the specimens already diagnosed, in order to control my diagnostic ability.

As was the case at the second examination, the observations recorded in the protocols were subjected to statistical analyses. An account of the results is given in subsequent chapters.

It was also possible to compare the protocols in the second (biased) and the third (unbiased) examinations. Some interesting features were brought to light. They are reported in Chapter 14.

CHAPTER 10

Results of the Unbiased Examination: The Diagnoses

The main object of the third (unbiased) examination was to endeavour to ascertain whether each particular specimen derived from a control animal or from one subjected to ECT, *i.e.*, to make a diagnosis. The specimens in question consisted of sections from both contralateral sides of the brain, *e.g.* the right and left gyrus lateralis, two sections from each side.

In making the diagnosis, the criteria enumerated earlier were relied upon to some extent. The final diagnosis did not, however, consist merely of an addition of the points allotted, but of a final evaluation in which the various observations were weighed against each other. All the phenomena which could indicate the diagnosis "shocked" were first sought for; the observations were then considered on the basis of the opposite hypothesis. The evaluations that were finally recorded were formulated as criticisms of the hypothesis that a certain specimen was taken from an animal subjected to ECT. The respective diagnoses were the following:

1. No grounds for diagnosis of shocked.
2. No definite grounds for diagnosis of shocked: both these diagnoses are denoted in the tables by 0;
3. No definite grounds for diagnosis of shocked, but possibly shocked: denoted by (0);
4. No definite grounds for diagnosis of shocked, but probably shocked: denoted by [(0)];
5. Presumably shocked: denoted by (S) and
6. Shocked: denoted by S.

E and (E), respectively, denote that other pathological phenomena of suspected encephalitic type, which could not be ascribed to ECT, were observed. In this connexion, E and (E) are therefore equivalent to 0.

A survey of the diagnoses made is given in Table 3.

Three pairs of specimens from each animal were examined, *i.e.*, sections from the right and left gyrus sigmoideus anterior, the right and left gyrus sigmoideus posterior and the right and left gyrus lateralis. Thus, three different diagnoses were assigned separately to each animal. Because there was no known mutual relationship between these diagnoses, they could be collocated.

Table 3

The Diagnoses

C = Controls			
Cat No.	Diagnosis		
	ant.	post.	lat.
9	0	0*	0
11	0	0	[(0)]
13	0*		0*
20	E	E	(S)
43	0	0	S
44	0	0	0
45	0	S	(0)
46	0	[(0)]	0
47	0	0	0
48	0	0	0
49	(E)	0	0
50	0	[(0)]	0
51	0	(0)	0
52	S	0	0
53	0	0	0
54	(S)	0	(0)

*one specimen.

A = 4 ECT's				
Cat. No.	Survival Time	Diagnosis		
		ant.	post.	lat.
26	24 hours	0	S	0
32	»	0	0	0
33	»	0	(0)	0
55	»	E	0	0
56	»	S	(S)	S
57	»	0	0	0
58	»	0	S	[(0)]
59	»	0	[(0)]	(S)
60	»	E	E	E
34	48 hours	0	0	0
35	»	0	0	0
65	»	0	S	0
66	»	S	(S)	S
67	»	S	S	S
68	»	E	(S)	0
36	96 hours	0	0	0
37	»	S	0	S
38	8 days	0	0	0
39	»	0	0	0
61	»	(S)	S	0
62	»	(S)	0	0
63	»	(S)	0	0
64	»	(E)	0	S

B = 11–16 ECT's				
Cat No.	Survival Time	Diagnosis		
		ant.	post.	lat.
28	48 hours	0	0	
29	»	S	(S)	(S)
27	96 hours	S	S	S
31	»	S	(0)	S
30	»	(S)	[(0)]	S
40	8 days	0	(S)	0
41	»	0	S	(0)
42	»	0	0	0

In this way, each individual animal could be given a collective diagnosis. For an animal to be diagnosed as shocked, at least two (S) or S were required, a single (S) or S being considered to imply a risk of faulty evaluation.

The following schema is a survey of the diagnoses for the individual animals; the statistical data recorded show the difference between the respective groups with regard to the diagnoses. C denotes the control animals and ECT the animals subjected to ECT; A: 4 shocks, B: 11—16 shocks. The figure after the classifying letter denotes the survival time (*cf.* p. 29).

Diagnosis	Groups	
	C	ECT_{A+B}
S	0	9
0	16	22

Diagnosis	Groups	
	C	ECT_A
S	0	5
0	16	18

	C	ECT_B
S	0	4
0	16	4

	C	$ECT_{B_{48+96}}$
S	0	4
0	16	1

	C	$ECT_{A_{48+96}+B_{48+96}}$
S	0	7
0	16	6

	$A_{24+8d}+B_{8d}$	$A_{48+96}+B_{48+96}$
S	2	7
0	16	6

Differences:

$C \sim A$	no significant difference
$C \sim B$	$p < 0.01$ (exact method: 0.0066)
$C \sim A+B$	$p < 0.02$ (exact method: 0.0190)
$C \sim B_{48+96}$	$p < 0.001$ (exact method: 0.0008)
$C \sim A_{48+96}+B_{48+96}$	$p < 0.01$ (exact method: 0.0011)
$A_{24+8d}+B_{8d} \sim A_{48+96}+B_{48+96}$	$p < 0.05$ (Mainland: 0.0141×2)

It thus appears possible, with the procedure used, to show a difference between the control animals and those subjected to ECT with regard to the neuropathological observations. In other words, it seems possible to diagnose the occurrence of cerebral changes associated with electric shock treatment. The material also revealed a tendency for the changes to be more apparent with a moderately long survival time (48—96 hours) than with a short (24 hours) or long (8 days) survival time.

If each pair of specimens is taken as the unit instead of the individual animals, the distribution of the diagnoses is as follows:

Diagnosis	No. of pairs of specimens in group					
	C	A	B	A+B	$A_{48+96}+B_{48+96}$	$A_{24+8d}+B_{8d}$
0 (0) [(0)]	42	48	11	59	18	41
S (S)	5	21	12	33	20	13

Differences:

$C \sim A$	no significant difference
$C \sim B$	$p < 0.001$ ($\chi^2 = 15.81$)
$C \sim A + B$	$p < 0.01$ ($\chi^2 = 8.74$)
$C \sim A_{48+96} + B_{48+96}$	$p < 0.001$ ($\chi^2 = 15.88$)
$A_{24+8d} + B_{8d} \sim A_{48+96} + B_{48+96}$ . .	$p < 0.01$ ($\chi^2 = 6.71$)

This procedure is, however, open to criticism. This is because three pairs of specimens were taken from each animal, and a mutual relationship between them might be expected to exist on the grounds of certain individual factors —such as sensitivity to the seizures, and age—common to the brain as a whole. For this reason, all the subsequent calculations were made on the animal as a unit.

The aforementioned mutual relationship between the different pairs of specimens from the same animal is illustrated by Table 3. It is evident from this table that, in the animals subjected to ECT, the same diagnosis—S and (S) collectively or 0, (0) and [(0)] collectively—for the three different parts of the brain occurred more frequently than there would be reason to expect in a random distribution of the diagnoses. The probability that the distribution found would be due to a random distribution is $p < 0.001$ ($t = 3.26$). This probability was tested by means of a test, based on dichotomization, elaborated by BLOMQVIST:[9]

$$S = \sum_{i=1}^{n} (y_i - P)^2$$

where the mean value

$$E(S) = n\,[p_1(1 - p_1) + p_2(1 - p_2) + p_3(1 - p_3)]$$

and the variance

$$\sigma^2(S) = \frac{4n^2}{n-1}[p_1(1 - p_1) \cdot p_2(1 - p_2) + p_1(1 - p_1) \cdot p_3(1 - p_3) + p_2(1 - p_2) \cdot p_3(1 - p_3)]$$

$$P = p_1 + p_2 + p_3$$

y_i = the sum of the figures in each row of the table, *i.e.*, the total number of S or (S) in the row.

p_i = the sum of the figures in each column in the table, *i.e.*, the number of S or (S) in the column, divided by the number of rows ($n = 31$).

$t = \dfrac{S - E(S)}{\sigma(S)}$ *i.e.*, t = the number of times its standard error that S deviates from its mean value.

Large values for S indicate that the diagnoses S or (S) would have been concentrated in a small number of animals, and small values for S indicate a distribution of the diagnoses among a large number of animals. It must be pointed out that the test is *one-sided.*

In the control animals, on the contrary, no such mutual relationship between the diagnoses is found, as is evident from even a cursory study of Table 3. The sporadic erroneous diagnoses are distributed at random among the control animals.

With respect to the animals subjected to ECT, the greater conformity of the diagnoses thus indicates that the changes observed in the different parts of the brain are to be attributed to some factor common to the brain as a whole. The most plausible supposition appears to be the ECT in combination with some individual factor.

Relationship of the Diagnoses to the Age

The question whether any relationship exists between a positive diagnosis and the age of the animal appears to warrant a special analysis. In the following schema, the younger animals have been compared with the older animals in the respective groups or sub-groups (controls and animals subjected to different series of ECT's.

	Group C (controls)	
	No. of younger animals	No. of older animals
Diagnosis S	0	0
Diagnosis 0 or E	8	8

	Group A (4 ECT's)	
	No. of younger animals	No. of older animals
Diagnosis S	0	5
Diagnosis 0 or E	11	7

	Group B (11—16 ECT's)	
	No. of younger animals	No. of older animals
Diagnosis S	1	3
Diagnosis 0 or E	4	0

	Groups A + B	
	No. of younger animals	No. of older animals
Diagnosis S	1	8
Diagnosis 0 or E	15	7

Sub-groups $A_{48+96} + B_{48+96}$
(animals with a medium survival time)

	No. of younger animals	No. of older animals
Diagnosis S	1	6
Diagnosis 0 or E	6	0

Differences younger animals:

$$\frac{\text{Diagnosis S}}{\text{Diagnosis 0 or E}}\ C \sim A = \frac{0}{8} \sim \frac{0}{11}$$

do. $C \sim B = \frac{0}{8} \sim \frac{1}{4}$

Differences older animals:

$$\frac{\text{Diagnosis S}}{\text{Diagnosis 0 or E}}\ C \sim A = \frac{0}{8} \sim \frac{5}{7}$$

do. $C \sim B = \frac{0}{8} \sim \frac{3}{0}\ p < 0.01$ (Mainland: 0.0061)

do. $C \sim A + B = \frac{0}{8} \sim \frac{8}{7}\ p < 0.02$ (exact method: 0.0193)

do. $C \sim A_{48+96} + B_{48+96} = \frac{0}{8} \sim \frac{6}{0}\ p < 0.001$ (exact method: 0.0003)

It may be deduced from the aforegoing that the differences between the neuropathological findings in the control animals and those subjected to ECT were found among the older animals.

There are two possible explanations. I might mistakenly have diagnosed changes due to age as changes due to ECT. Or, older animals might be, on the whole, more susceptible to cerebral changes in the course of ECT. The following fact argues against the first alternative. Although there was no difference between the control animals and those subjected to ECT with respect to the estimated age*, none of the former category were diagnosed as shocked. In other words, this speaks in favour of the second alternative. I might also have combined possible changes due to age with changes caused by ECT; in this case only the somewhat older animals would have exceeded the threshold dose above which the diagnosis S was applicable. It therefore appeared warranted to make a closer analysis of the various diagnostic criteria in view of their reliability as a basis for differentiating between controls and animals subjected to ECT, as well as their relationship to the age.

* Of the five specimens from the control animals that were erroneously diagnosed as (S) or S, 2 were from animals in the younger group and 3 from those in the older age group.

The Different Neuropathological Phenomena

a. CHANGES IN THE VESSEL WALL

The changes in the vessel wall in each specimen were graded on the basis of the evaluation at the third (unbiased) examination. Four grades were used: 0, 1, 2 and 3 (and, in a few cases, half-grades). Each animal was thus assigned a total of six values, corresponding to the six specimens examined (right and left gyrus sigmoideus anterior, right and left gyrus sigmoideus posterior and right and left gyrus lateralis). For the individual animal, the total could thus vary between 0 and 18.*

The values found are recorded in Table 4. The agreement with a normal distribution within the different groups appeared to warrant a t analysis. It showed the following differences:

$C \sim A$	$0.02 < p < 0.05$ $(t = 2.10)$
$C \sim B$	$p < 0.01$ $(t = 3.01)$
$C \sim A + B$	$p < 0.01$ $(t = 2.91)$
$C \sim A_{48+96} + B_{48+96}$	$p < 0.01$ $(t = 3.40)$
$A_{24+8d} + B_{8d} \sim A_{48+96} + B_{48+96}$	$0.02 < p < 0.05$ $(t = 2.37)$

There is thus a difference between the animals subjected to ECT and the controls with regard to the changes in the vessel wall. This difference is most marked in a comparison between the controls and the animals subjected to more intense treatment, the significance of the difference being $p < 0.01$, and between the controls and the animals with a survival time of medium duration, the significance of the difference being $p < 0.01$.

There was also a tendency for the treated animals with a moderately long survival time to exhibit more changes than those with a short or long survival time, respectively.

The question raised in Chapter 10 of the relationship of the reactive changes in the vessel wall to the age of the animal is illuminated by Table 5 A. In it, each group of animals subjected to ECT has been classified according to age: the younger animals in one group and the older in another, as in Chapter 4 (*cf.* p. 29).

The number, the mean value and the standard deviation are shown in Table 5 B and the differences in Table 5 C.

There is thus no difference between the group of younger and older control

* In the few cases in which a value was missing, the total was reached by estimating the missing value in proportion to those found.

Table 4

Changes in the Vessel Wall

C = Controls	
Cat No.	Points
9	2.5
11	6
13	9
20	10
43	11
45	9
46	7
44	5
47	6
48	12
49	4
50	8
51	8
52	7
53	3
54	7
	114.5

$n = 16$
$\bar{x} = 7.156$
$s = 2.718$

A = 4 ECT's	
Cat No.	Points
26	9.5
33	8
32	6
55	2.5
56	12
57	4
58	9
59	10
60	10
35	11
34	6
65	10
66	16
67	18
68	12
36	7
37	11
38	8
39	5
61	11
62	9
63	7
64	11
	213

$n = 23$
$\bar{x} = 9.261$
$s = 3.549$

B = 11 – 16 ECT's	
Cat No.	Points
28	4.5
29	16
27	18
31	12
30	13
40	11
41	13
42	9
	96.5

$n = 8$
$\bar{x} = 12.063$
$s = 4.143$

Groups A + B:
$n = 31$
$\bar{x} = 9.984$
$s = 3.846$

Sub-groups A_{48+96} + B_{48+96}:
$n = 13$
$\bar{x} = 11.885$
$s = 4.360$

animals, respectively, with regard to the phenomenon of changes in the vessel wall; on the contrary, the agreement is good. Nor is there any significant difference in this respect between the corresponding groups of treated animals, although a slight tendency ($p < 0.10$) can be discerned. This argues against the supposition that age alone might play any decisive rôle in the occurrence of the changes in question. On the other hand, it is evident that—with regard to this phenomenon—the differences between the control animals and those subjected to ECT are considerably more marked in the group of older animals, in which the difference lies below the significance level $p < 0.01$, whereas in the younger group the difference is not significant. The most plausible explana-

Table 5 A

Changes in the Vessel Wall / Age of the Animals

C: younger	
Cat No.	Points
11	6
20	10
45	9
46	7
47	6
48	12
49	4
53	3
	57

C: older	
Cat No.	Points
9	2.5
13	9
43	11
44	5
50	8
51	8
52	7
54	7
	57.5

A: younger	
Cat No.	Points
26	9.5
32	6
55	2.5
57	4
58	9
35	11
34	6
65	10
68	12
36	7
64	11
	88

A: older	
Cat No.	Points
33	8
56	12
59	10
60	10
66	16
67	18
37	11
38	8
39	5
61	11
62	9
63	7
	125

B: younger	
Cat No.	Points
28	4.5
29	16
40	11
41	13
42	9
	53.5

B: older	
Cat No.	Points
27	18
31	12
30	13
	43

tion seems to be that a higher age may, to some extent, have contributed to the occurrence of these changes in association with ECT. It nevertheless appears feasible to attribute the changes in the vessel wall, as they have been defined and observed, mainly to the electric shock treatment.

Table 5 B

Changes in the Vessel Wall / Age of the Animals

Group	Code	n	$\bar{x}$	s
Controls: younger	C_y	8	7.125	3.044
Controls: older	C_o	8	7.188	2.563
4 ECT's: younger	A_y	11	8.000	3.106
4 ECT's: older	A_o	12	10.417	3.655
11—16 ECT's: younger	B_y	5	10.700	4.324
11—16 ECT's: older	B_o	3	14.333	3.214
	$A_y + B_y$	16	8.884	3.618
	$A_o + B_o$	15	11.200	3.821
$A_{48+96} + B_{48+96}$: younger		7	9.500	3.969
$A_{48+96} + B_{48+96}$: older		6	14.667	3.077

Table 5 C

Changes in the Vessel Wall / Age of the Animals: Differences

Group	C_y	C_o	A_y	A_o	B_y	$A_y + B_y$	$A_{48+96}+B_{48+96y}$
C_o	0.063 $p > 0.9$						
A_y	0.875 $0.50 < p < 0.60$						
A_o		3.229 $0.02 < p < 0.05$	2.417 $0.10 \leq p < 0.20$				
B_y	3.575 $0.10 < p < 0.20$		2.700				
B_o		7.145 $(p < 0.01)$		3.916	3.633 $(0.20 < p < 0.30)$		
A_y+B_y	1.759 $0.20 < p < 0.30$						
A_o+B_o		4.012 $p < 0.01$				2.316 $0.05 < p \leq 0.10$	
$A_{48+96}+B_{48+96y}$	2.375 $0.20 < p < 0.30$						
$A_{48+96}+B_{48+96o}$		7.479 $(p < 0.01)$					5.167 $(0.02 < p < 0.05)$

b. PIGMENT IN THE PERIVASCULAR SPACES

In some cases I observed small particles of yellow pigment lying in the dilated, sac-like perivascular spaces. More infrequently, it was found phagocytozed in the histiocytes present at this site. This phenomenon was recorded separately at the third, unbiased examination. A collocation of the specimens from the gyrus sigmoideus anterior, the gyrus sigmoideus posterior and the gyrus lateralis gave the impression that such pigment was more often present in the brains of the animals subjected to ECT than in those of the controls.

A statistical analysis also showed that the incidence of such pigment was higher among the animals subjected to ECT and with a medium survival time (Groups $A_{48+96} + B_{48+96}$) than among the controls. It disclosed a similar difference between the treated animals with a medium survival time and those with a short or long survival time, respectively (Groups $A_{24+8d} + B_{8d}$) as is evident from the following survey:

	No. of animals		
	Controls	$A_{48+96} + B_{48+96}$	$A_{24+8d} + B_{8d}$
Pigment	3	9	2
No pigment	13	4	16

Differences:

$C \sim A_{48+96} + B_{48+96}$ . . . . . $p < 0.01$ (exact method: 0.0095)

$A_{24+8d} + B_{8d} \sim A_{48+96} + B_{48+96}$ . . . $p < 0.01$ (exact method: 0.0020)

On the other hand, it was not possible to show any statistical difference between the older and younger animals in this respect, although a slight tendency could be discerned to a more frequent occurrence of pigment in the older animals.

Later, I endeavoured to ascertain the nature of this pigment. I found that it did not stain with the Turnbull method according to Tirmann & Schmelzer (cited by ROMEIS[86]). The Gmelin reaction was also negative, but the pigment could be stained with Gram's gentian violet solution (von Volkmann's method). The combined results indicate that the pigment was not hæmatogenic but of the autogenic type. (On the other hand, the presence of hæmatogenic pigment as well, as a result of ECT, cannot be ruled out on the basis of this investigation.)

The occurrence of autogenic pigment in the nerve cells can scarcely be accorded any pathological importance in this connexion. This statement does not, however, apply to the presence of such pigment in the perivascular spaces or in phagocytes. There is namely reason to suppose that it is not formed at these sites, but that it develops on disintegration of nerve cells and is then transported to the vessels. Pictures illustrating this course of events may sometimes be seen. These illustrations, together with the conspicuous accu-

mulation of such pigment at this site in animals subjected to ECT shortly before death (48—96 hours), strongly indicate that the occurrence of perivascular pigment of the autogenic type is mainly to be attributed to the effect of ECT. The occurrence of such pigment in the perivascular spaces is not, however, a reliable criterion for distinguishing, at an unbiased examination, between control animals and those subjected to ECT.

c. ACUTE CEREBRAL ŒDEMA

The width of the perivascular spaces of the larger vessels varied from one specimen to the next and even from one blood vessel to the next. Although I realized that this variation must be associated with the degree of shrinkage of the specimen, I considered that it might also be of importance as a measure of acute œdema. An account is given in the following of an attempt to evaluate the occurrence and degree of acute œdema against the background of both these factors.

At the third (unbiased) examination, the following five grade scale was used for the evaluation of the perivascular spaces of the larger vessels.

0. No dilatation of the perivascular spaces.

1. No dilatation in the majority of the perivascular spaces, sporadic slight dilatation.

2. No dilatation in the minority of perivascular spaces, slight dilatation in the majority.

3. In addition to 2, sporadic dilatation of a higher degree.

4. As in 3, but more widespread, marked dilatation.

No significant differences could be shown between the various groups in this respect. There was only a tendency to such ($p < 0.05$) between the controls and the group of animals given the largest number of ECT's (Group B) if the dividing line was drawn between 0 and the other classes.

Dilatation of the perivascular spaces of the larger vessels may be due to shrinkage—as already pointed out—but it may also possibly be interpreted as an indication of cerebral œdema. In every case in which it was observed, it was in the form of simple dilatation with no signs of a vessel wall reaction, *i.e.*, it appeared to be a purely passive dilatation. The phenomenon was thus of little specificity, and if it is to be taken as a sign of cerebral œdema, it must have been of the fresh, terminal type. It might therefore be interpreted as a result of the ether anesthesia and the operation. The preceding ECT could then be envisaged as having, more or less transiently, decreased the resistance of the blood vessels. This interpretation would then be on a parallel with that of the occurrence of terminal hæmorrhages.

The phenomenon described in the aforegoing was thus considerably less specific than the changes in the vessel wall, which appeared to have a direct

association with the ECT and in which the histological picture was definitely more characteristic (*cf.* page 44).

Finally, it must be emphasized that an evaluation of possible acute cerebral œdema on the basis of the dilatation of the perivascular spaces of the larger vessels after fixation of the tissues is obviously very uncertain. General conclusions are only warranted when the changes are more marked than was the case in this material. This also implies that, with the method used, the occurrence of *slight* acute œdema cannot be ruled out with any degree of certainty.

d. HÆMORRHAGES

As stated in an earlier chapter, a routine search for hæmorrhages in the cerebral parenchyma was made at both the macroscopical and the microscopical examinations. The latter also permitted an estimation of the age of the hæmorrhages.

With two exceptions (Cats nos. 26 and 66) all the hæmorrhages were fresh, judging by the unchanged shape of the corpuscles (see Fig. 13). No homogeinization or attempts at organization, such as an invasion by histiocytic elements or a glial reaction in the vicinity of the hæmorrhage, were present nor could any free hæmatogenic pigment be observed. It was therefore concluded that the hæmorrhages were fresh and terminal, presumably arising during the operation on the head and the brain which was performed immediately before sacrificing the animals.

Except in one case (Cat no. 26) the hæmorrhages were small and petechial, of the small extravasation type or slightly larger. They were often multiple, the site of predilection being the frontal region, although they were also observed in the temporal region.

They were observed in the brain both of the control animals and of those subjected to ECT. There was, however, a difference in the incidence in the two categories.

At the third examination, a figure varying between 0 and 6 was recorded for each animal, denoting the number of specimens out of the six examined (from the gyrus sigmoideus anterior, gyrus sigmoideus posterior and gyrus lateralis of each hemisphere) in which hæmorrhages were found. These figures are shown in Table 6.

If the lowest category, *i.e.*, the animals with no observed hæmorrhages, is compared with the other categories, the following differences are obtained:

$$C \sim A + B: \frac{7}{9} \sim \frac{3}{28} \quad \ldots\ldots\ldots \quad p < 0.02 \text{ (exact method: 0.0112)}$$

$$C \sim A_{48+96} + B_{48+96}: \frac{7}{9} \sim \frac{0}{13} \quad \ldots\ldots \quad p < 0.01 \text{ (exact method: 0.0084)}$$

whereas if the two lowest categories, *i.e.*, no specimen or only one containing

Table 6

Hæmorrhages

No. of Specimens with Hæmorrhages	No. of Animals		
	Controls	A + B	$A_{48+96} + B_{48+96}$
0	7	3	
1	7	11	4
2		10	5
3		3	1
4	2	4	3
5			
6			

Differences:

$C \sim A + B$: $\frac{7}{9} \sim \frac{3}{28}$ . . . . . $p < 0.02$ (exact method: 0.0112)

$C \sim A_{48+96} + B_{48+96}$: $\frac{7}{9} \sim \frac{0}{13}$ . . . . . $p < 0.01$ (exact method: 0.0084)

or

$C \sim A + B$: $\frac{14}{2} \sim \frac{14}{17}$ . . . . . $p < 0.02$ (exact method: 0.0107)

$C \sim A_{48+96} + B_{48+96}$: $\frac{14}{2} \sim \frac{4}{9}$ . . . . . $p < 0.01$ (exact method: 0.0027)

a hæmorrhage, are compared with the others (hæmorrhages in several specimens) the differences are the following:

$C \sim A + B$: $\frac{14}{2} \sim \frac{14}{17}$ . . . . . $p < 0.02$ (exact method: 0.0107)

$C \sim A_{48+96} + B_{48+96}$: $\frac{14}{2} \sim \frac{4}{9}$ . . . . $p < 0.01$ (exact method: 0.0027)

Terminal hæmorrhages thus appear to occur more frequently in the animals subjected to ECT than in the controls and the probability of this being purely a random variation is approximately $p = 0.01$. On the other hand, the histological evaluation showed that only in exceptional cases could the hæmorrhages be assumed to have occurred in the course of the ECT. The most plausible explanation appears to be that the electric shocks, in combination with some individual factor, resulted in an increased tendency to terminal cerebral hæmorrhages.

Incidence of Hæmorrhages in Younger and Older Animals, Respectively

It is evident from Table 7 that the incidence of terminal hæmorrhages was lowest in the younger control animals. The difference found earlier between the controls and the treated animals could still be noted in the younger group ($p < 0.01$) after a classification into younger and older animals. In the older

Table 7

Hæmorrhages / Age of the Animals

No. of Specimens with Hæmorrhages	No. of Animals			
	Controls		A + B	
	younger	older	younger	older
0	6	1	2	1
1	1	6	6	5
2			6	4
3			2	1
4	1	1		4
5				
6				

Differences:

$C_y \sim C_o$: $\frac{6}{2} \sim \frac{1}{7}$ . . . . . $p < 0.05$ (Mainland: 0.0203 × 2)

$C_y \sim A_y + B_y$: $\frac{6}{2} \sim \frac{2}{14}$ . . . . . $p < 0.01$ (Mainland: 0.0048 × 2)

group, hæmorrhages were more frequently present in both categories of animals. A difference ($p < 0.05$) was also found between the younger and the older control animals, respectively.

There thus appeared to be a tendency for both the ECT and the higher age to predispose towards the occurrence of terminal cerebral hæmorrhages.

*

In the whole material, vasodilatation was consistently found in the specimens from the cerebral cortex; the vessels were dilated and filled with blood. This argues in favour of the occurrence of cerebral terminal hyperæmia, which may presumably be attributed to the ether anesthesia.

e. GLIAL REACTION IN THE PARENCHYMA

In the present study, glial reaction is used as a common term to denote the reaction in the immediate vicinity of the nerve cells (satellitosis) and in the remainder of the cerebral parenchyma. At the unbiased examination, the reaction was judged according to a three-grade scale: none or very slight, slight, and marked. The first two grades were those most usually observed, the third grade being found only sporadically (3 out of the total 282 recorded, all in group A). The first grade was denoted as 0, the second as 1 and the third as 2. The value for each animal was composed of the total of the values allotted to each of the six specimens examined; it could thus vary between 0 and 12 but in actual fact never exceeded 7.

The values obtained are recorded in Table 8 A*. A t analysis showed the following differences.

$C \sim A$	p approx. 0.02 ($t = 2.44$)
$C \sim B$	$0.01 < p < 0.02$ ($t = 2.57$)
$C \sim A + B$	$p < 0.01$ ($t = 3.09$)
$C \sim A_{48+96} + B_{48+96}$	$p < 0.01$ ($t = 2.91$)
$A_{24+8d} + B_{8d} \sim A_{48+96} + B_{48+96}$	$0.20 < p < 0.30$ ($t = 1.24$)

Table 8 A

Glial Reaction in the Parenchyma

C = Controls	
Cat No.	Points
9	0
11	0.5
13	0
20	3
43	3.5
45	0.5
46	0.5
44	0
47	0.5
48	0
49	0
50	0.5
51	0.5
52	1
53	0
54	2.5
	13

$n = 16$
$\bar{x} = 0.813$
$s = 1.138$

A = 4 ECT's	
Cat No.	Points
26	2
33	0
32	0.5
55	0
56	4
57	0
58	3
59	2
60	1
35	3
34	0.5
65	1
66	3
67	7
68	1
36	0.5
37	3
38	0.5
39	1
61	5.5
62	1.5
63	2
64	3
	45

$n = 23$
$\bar{x} = 1.956$
$s = 1.803$

B = 11–16 ECT's	
Cat No.	Points
28	0
29	5
27	4.5
31	3
30	3
40	2.5
41	4
42	0
	22

$n = 8$
$\bar{x} = 2.75$
$s = 1.889$

* In those cases in which a single value was missing, the total was computed in proportion to the values found.

A study of Table 8 A nevertheless discloses a considerably skew distribution of the material, thus decreasing the reliability of the *t* analysis. The distribution of the values according to the classes is shown in Table 8 B.

Table 8 B

Glial Reaction in the Parenchyma

Classes	No. of Animals in Group			
	C	A	B	$A_{48+96} + B_{48+96}$
0	6	3	2	1
≦ 1	7	8		4
≦ 2		4		
≦ 3	2	5	3	5
≦ 4	1	1	1	
≦ 5			2	2
≦ 6		1		
≦ 7		1		1
≦ 12				

Differences:

$C \sim B: \frac{13}{3} \sim \frac{2}{6}$ . . . . . $p < 0.05$ (exact method: 0.0215)

$C \sim A + B: \frac{13}{3} \sim \frac{13}{18}$ . . . . . $p < 0.02$ (exact method: 0.0139)

$C \sim A_{48+96} + B_{48+96}: \frac{13}{3} \sim \frac{5}{8}$ . . . . . $p < 0.05$ (Mainland: 0.0234×2)

Judging by Table 8 B there is thus a difference ($p < 0.02$) in this respect between the control animals and those subjected to ECT.

However, a study of the material after a classification into age groups shows that the incidence of glial reactions was highest in the older group of shocked animals. There is no evident difference between the younger and older control animals (see Tables 9 A, B, C and D). The difference in this respect between the control animals and those subjected to ECT is most apparent in the older group. This fact indicates that increasing age may be a predisposing factor in the occurrence of glial reactions associated with ECT. Moreover, this increased predisposition at a higher age is more marked in the case of glial reactions than in that of the phenomenon of changes in the vessel wall (*cf.* page 53).

Table 9 A

Glial Reaction in the Parenchyma / Age of the Animals

C: younger	
Cat No.	Points
11	0.5
20	3
45	0.5
46	0.5
47	0.5
48	0
49	0
53	0
	5

C: older	
Cat No.	Points
9	0
13	0
43	3.5
44	0
50	0.5
51	0.5
52	1
54	2.5
	8

A: younger	
Cat No.	Points
26	2
32	0.5
55	0
57	0
58	3
35	3
34	0.5
65	1
68	1
36	0.5
64	3
	14.5

A: older	
Cat No.	Points
33	0
56	4
59	2
60	1
66	3
67	7
37	3
38	0.5
39	1
61	5.5
62	1.5
63	2
	30.5

B: younger	
Cat No.	Points
28	0
29	5
40	2.5
41	4
42	0
	11.5

B: older	
Cat No.	Points
27	4.5
31	3
30	3
	10.5

Table 9 B

Glial Reaction in the Parenchyma / Age of the Animals

Group	Code	n	$\bar{x}$	s
Controls: younger	C_y	8	0.625	0.991
Controls: older	C_o	8	1.000	1.309
4 ECT's: younger	A_y	11	1.318	1.210
4 ECT's: older	A_o	12	2.542	2.094
11–16 ECT's: younger	B_y	5	2.300	2.280
11–16 ECT's: older	B_o	3	3.500	0.866
	$A_y + B_y$	16	1.625	1.607
	$A_o + B_o$	15	2.733	1.926
$A_{48+93} + B_{48+96}$: younger		7	1.571	1.789
$A_{48+96} + B_{48+96}$: older		6	3.917	1.625

Table 9 C

Glial Reaction in the Parenchyma / Age of the Animals: Differences

Group	C_y	C_o	A_y	A_o	B_y	A_y+B_y	$A_{48+96}+B_{48+96y}$
C_o	0.375 ($0.50 < p < 0.60$)						
A_y	0.693						
A_o		1.542 ($0.05 < p < 0.10$)					
B_y	1.675 ($0.10 < p < 0.20$)		0.982				
B_o		2.500 ($p < 0.01$)		0.958			
A_y+B_y	1.000 ($0.05 < p < 0.10$)						
A_o+B_o		1.733 ($0.01 < p < 0.02$)				1.108 ($0.05 < p < 0.10$)	
$A_{48+96}+B_{48+96y}$	0.946						
$A_{48+96}+B_{48+96o}$		2.917 ($p < 0.01$)					2.346 ($0.02 < p < 0.05$)

Table 9 D

Glial Reaction in the Parenchyma / Age of the Animals

Classes	No. of Animals					
	C		A + B		A_{48+96} + B_{48+96}	
	younger	older	younger	older	younger	older
0	3	3	4	1	1	
≦ 1	4	3	5	3	4	
≦ 2			1	3		
≦ 3	1	1	4	4	1	4
≦ 4		1	1	1		
≦ 5			1	1	1	1
≦ 6				1		
≦ 7				1		1
≦ 12						

Differences:

$C_o \sim A_o + B_o$: $\frac{6}{2} \sim \frac{4}{11}$ . . . . . $p < 0.05$ (exact method: 0.0393)

$C_o \sim A_{48+96} + B_{48+96}$ older: $\frac{6}{2} \sim \frac{0}{6}$ . . . . . $p < 0.01$ (exact method: 0.0097)

$C_y \sim A_o + B_o$: $\frac{7}{1} \sim \frac{4}{11}$ . . . . . $p < 0.01$ (exact method: 0.0094)

$C_y \sim A_{48+96} + B_{48+96}$ older: $\frac{7}{1} \sim \frac{0}{6}$ . . . . . $p < 0.01$ (exact method: 0.0047)

f. SATELLITOSIS

Satellitosis may be said to constitute a partial phenomenon of the glial reaction. The latter has been analyzed in the preceding section. The glial reaction is, however, on broad lines a phenomenon more difficult to assess than satellitosis which, because it is more sharply delimited, is easier to observe and to grade. At the third (unbiased) examination, satellitosis was evaluated according to a four-grade scale (0, 1, 2, 3) in the same way as the changes in the vessel wall. Each animal was thus allotted a total of six values, corresponding to the six specimens from the respective parts of the brain; the total could vary between 0 and 18.

The distribution of the values is shown in Table 10. The agreement with a normal distribution within the various groups appeared to be sufficiently satisfactory to warrant a *t* analysis. The following differences were found.

$C \sim A$ . . . . . $0.05 < p < 0.10$ ($t = 1.71$)

$C \sim B$ . . . . . $0.01 < p < 0.02$ ($t = 2.65$)

$C \sim A + B$ . . . . . $0.02 < p < 0.05$ ($t = 2.35$)

$C \sim A_{48+96} + B_{48+96}$ . . . . . $p < 0.01$ ($t = 3.23$)

$A_{24+8d} + B_{8d} \sim A_{48+96} + B_{48+96}$ . . . p approx. 0.05 ($t = 2.05$)

Table 10

Satellitosis

C = Controls	
Cat No.	Points
9	6
11	6
13	12
20	3
43	12
45	4
46	7
44	10
47	5
48	12
49	6
50	10
51	11
52	10
53	5
54	8
	127

$n = 16$
$\bar{x} = 7.938$
$s = 3.065$

A = 4 ECT's	
Cat No.	Points
26	8.5
33	9
32	8
55	3
56	14
57	3
58	13
59	14
60	8
35	11
34	7
65	10
66	13
67	15
68	9
36	11
37	14
38	8
39	4
61	13
62	10
63	10
64	10
	225.5

$n = 23$
$\bar{x} = 9.804$
$s = 3.807$

B = 11–16 ECT's	
Cat No.	Points
28	6
29	16
27	14
31	12
30	13
40	10
41	12
42	9
	92

$n = 8$
$\bar{x} = 11.500$
$s = 3.116$

Groups A + B:

$n = 31$
$\bar{x} = 10.242$
$s = 3.392$

Sub-groups A_{48+96} + B_{48+96}:

$n = 13$
$\bar{x} = 11.615$
$s = 3.015$

There is thus a difference between the animals subjected to ECT and the controls with respect to the phenomenon of satellitosis. The difference is most marked in a comparison between the controls and the treated animals with a medium survival time ($p < 0.01$) and between the controls and the animals subjected to more intense treatment ($p < 0.02$). There is also a tendency for the animals subjected to ECT and with a medium survival time to exhibit a higher degree of satellitosis than the treated animals with a short or long survival time, respectively.

The relationship of satellitosis to the age of the experimental animals is illustrated by Table 11 A. The values, mean values and the standard deviations are shown in Table 11 B and the differences in Table 11 C.

Table 11 A

Satellitosis / Age of the Animals

C: younger	
Cat No.	Points
11	6
20	3
45	4
46	7
47	5
48	12
49	6
53	5
	48

C: older	
Cat No.	Points
9	6
13	12
43	12
44	10
50	10
51	11
52	10
54	8
	79

A: younger	
Cat No.	Points
26	8.5
32	8
55	3
57	3
58	13
35	11
34	7
65	10
68	9
36	11
64	10
	93.5

A: older	
Cat No.	Points
33	9
56	14
59	14
60	8
66	13
67	15
37	14
38	8
39	4
61	13
62	10
63	10
	132

B: younger	
Cat No.	Points
28	6
29	16
40	10
41	12
42	9
	53

B: older	
Cat No.	Points
27	14
31	12
30	13
	39

A significant difference ($p < 0.01$) is found between the groups of older and younger control animals, respectively, indicating that age as such plays a considerable rôle in the occurrence of the phenomenon in question. Despite this fact, a definite tendency for the animals subjected to ECT to exhibit more satellitosis than the controls is still evident after a classification according to age.

Table 11 B

Satellitosis / Age of the Animals

Group	Code	n	$\bar{x}$	s
Controls: younger	C_y	8	6.000	2.726
Controls: older	C_o	8	9.875	2.031
4 ECT's: younger	A_y	11	8.500	3.170
4 ECT's: older	A_o	12	11.000	3.358
11–16 ECT's: younger	B_y	5	10.600	3.715
11–16 ECT's: older	B_o	3	13.000	1.000
	$A_y + B_y$	16	9.156	3.375
	$A_o + B_o$	15	11.400	3.112
$A_{48+96} + B_{48+96}$: younger		7	10.000	3.266
$A_{48+96} + B_{48+96}$: older		6	13.500	1.054

Table 11 C

Satellitosis / Age of the Animals: Differences

Group	C_y	C_o	A_y	A_o	B_y	A_y+B_y	$A_{48+96}+B_{48+96y}$
C_o	3.875 $p < 0.01$						
A_y	2.500 $0.05 < p < 0.10$						
A_o		1.125					
B_y	4.600 $(0.02 < p < 0.05)$		2.100				
B_o		3.125 $(p < 0.01)$		2.000			
A_y+B_y	3.156 $0.02 < p < 0.05$						
A_o+B_o		1.525 $0.10 < p < 0.20$				2.244 $0.05 < p < 0.10$	
$A_{48+96}+B_{48+96y}$	4.000 $0.02 < p < 0.05$						
$A_{48+96}+B_{48+96o}$		3.625 $(p < 0.001)$					3.500 $(0.02 < p < 0.05)$

g. CHANGES IN THE NERVE CELLS

The reaction of the nerve cells to various noxæ is the most vital problem in neuropathology. It is also a difficult and complicated matter. This is because it is necessary to take into consideration a number of artefacts, such as the effect of the fixative, variations in the staining and in the thickness of the sections. (These problems will be discussed in a later chapter.) An additional difficulty is that subjective evaluation and interpretation of the appearance of the nerve cells have for long been the only means for research in this field. This method is still that most commonly used and has been applied in the present investigation. It is therefore evident that the results of such studies must be regarded as somewhat unreliable, at any rate insofar as changes of a less marked nature are concerned.

The changes that may be observed after ECT are fairly subtle; this is apparent from the survey of the literature in Part One and is confirmed by the present study. Such phenomena as changes in the vessel wall and glial reactions are, however, easier to observe than nerve cell changes, because they are more distinct. A considerable risk of bias is nevertheless evident even with respect to changes in the vessel wall, if the examiner is aware of the origin of the specimens. This source of error will be discussed in Chapter 14.

The risk of faulty and biased evaluation is still greater in the case of the nerve cell changes in question, since they are less distinct.

At the first examination I was aware of the origin of the specimens. I thought that I could discern a tendency to chromophobia in general, at least in a considerable number of medium-sized pyramidal cells, in the animals with a short survival time after ECT. The second examination (also biased) appeared to confirm my observation. It was duly noted in the protocols. A subsequent statistical analysis should have afforded proof of the difference in chromaffinity between the control animals and those subjected to ECT—had the observations not been biased. The figures obtained will not be published here. I found it necessary to resort to a third, unbiased examination. *At the third, unbiased, examination* of the specimens from the gyrus sigmoideus anterior, gyrus sigmoideus posterior and gyrus lateralis, I also recorded the estimation of chromophobia of the nerve cells "in general". This denoted the collective assessment of the nerve cells in the relevant specimen. A subsequent statistical analysis revealed that I had been unable to grade the chromophobia "in general" in such a way that any difference whatsoever could be shown between the control animals and those subjected to ECT. Moreover, the large majority of the specimens were denoted as chromoneutral "in general".

The conditions differed somewhat if other degrees of chromaffinity and the distribution of the chromophobic cells were also taken into consideration. Thus, nuclear hyperchromatism with coincident peripheral chromophobia

could be noted in some cells. Such cells presumably correspond to Einarson's group 2 (*cf.* p. 39). He included them in the chromophobic group, since their cytoplasm is chromophobic and shows slight—somewhat homogeneous—stainability with less marked tigroid substance. They nevertheless differ from those nerve cells which are chromophobic throughout, in that the nucleus exhibits hyperchromatism.

Thus, the nerve cells of the animals subjected to ECT appeared to show a greater *variability* with regard to chromaffinity than those of the controls. In other words, in the former category, chromophobic cells—either with or without nuclear hyperchromatism—were present concurrently with the normal (chromoneutral) cells (see Fig. 19). Moreover, this variability seemed to be accentuated in some areas, especially in the deeper layers (Vth and VIth). Thus, small foci with cells of varying chromaffinity were observed: the most chromophobic cells were often in the centre and those with nuclear hyperchromatism in the periphery.

Because such variability was more easily discernible, it was recorded separately, the following grades being used. 0: no or very slight variability; 1: some variability; 2: marked variability. At the third examination, six specimens from each animal were studied: from the right and left gyrus sigmoideus anterior, the right and left gyrus sigmoideus posterior and the right and left gyrus lateralis. Each animal thus received a total of six values, which could vary between 0 and 12; they are recorded in Table 12. Although there was a considerable skewness in the distribution, a t analysis seemed to be not entirely unwarranted. The following differences were found.

$C \sim A$ . . . . . $0.02 < p < 0.05$ ($t = 2.25$)
$C \sim B$ . . . . . $p < 0.01$ ($t = 3.02$)
$C \sim A + B$ . . . . . $p < 0.01$ ($t = 3.16$)
$C \sim A_{48+96} + B_{48+96}$ . . . . . $p < 0.01$ ($t = 2.83$)
$A_{24+8d} + B_{8d} \sim A_{48+96} + B_{48+96}$ . . . $0.10 < p < 0.20$ ($t = 1.33$)

The contingency table below also throws light on the differences.

	C	A + B
Points: 0	10	6
Points: 1—12	6	25

$C \sim A + B$: $p < 0.01$ ($\chi^2 = 8.18$; exact method: 0.0047)

$C \sim B$: $\frac{10}{6} \sim \frac{1}{7}$: $p < 0.05$ (exact method: 0.0335)

$C \sim A_{48+96} + B_{48+96}$: $\frac{10}{6} \sim \frac{2}{11}$: $p < 0.05$ (exact method: 0.0216)

It is therefore justified to conclude that there is a significant difference ($p < 0.01$) between the control animals and those subjected to ECT with regard to the phenomenon of variability of the nerve cells.

Table 12

Variability of the Nerve Cells

C = Controls	
Cat No.	Points
9	0
11	1
13	2
20	0
43	3.5
45	0
46	0
44	0
47	0
48	2.5
49	0
50	0
51	0
52	6
53	0
54	4
	19

$n = 16$
$\bar{x} = 1.188$
$s = 1.879$

A = 4 ECT's	
Cat No.	Points
26	2
33	3
32	4
55	0
56	6
57	2
58	0
59	6
60	0
35	4
34	0
65	1
66	4
67	9
68	0
36	2
37	5.5
38	1
39	2
61	3
62	4
63	3
64	1
	62.5

$n = 23$
$\bar{x} = 2.717$
$s = 2.369$

B = 11–16 ECT's	
Cat No.	Points
28	1
29	10
27	10
31	8
30	3
40	5
41	9
42	0
	46

$n = 8$
$\bar{x} = 5.750$
$s = 4.062$

Groups A + B:

$n = 31$
$\bar{x} = 3.500$
$s = 3.127$

Sub-groups A_{48+96} + B_{48+96}:

$n = 13$
$\bar{x} = 4.423$
$s = 3.741$

The relationship of this phenomenon to the age of the animals is shown in Tables 13 A, 13 B, 13 C and 13 D. It may be inferred from these tables that the aforementioned difference is considerably less apparent after a classification of the material into older and younger animals. The figures as such nevertheless indicate that ECT plays a greater rôle in the occurrence of variability of the nerve cells than does age, but that the latter appears to predispose towards the occurrence of this phenomenon. The difference is most marked when these two factors, *i.e.*, age and ECT, are combined.

$$C_{younger} \sim (A + B)_{older}: \frac{6}{2} \sim \frac{1}{14}: p < 0.01 \text{ (Mainland: } 0.0017 \times 2)$$

$$C_{younger} \sim (A_{48+96} + B_{48+96})_{older}: \frac{6}{2} \sim \frac{0}{6}: p < 0.01 \text{ (exact method: } 0.0097)$$

Table 13 A

Variability of the Nerve Cells / Age of the Animals

C: younger	
Cat No.	Points
11	1
20	0
45	0
46	0
47	0
48	2.5
49	0
53	0
	3.5

C: older	
Cat No.	Points
9	0
13	2
43	3.5
44	0
50	0
51	0
52	6
54	4
	15.5

A: younger	
Cat No.	Points
26	2
32	4
55	0
57	2
58	0
35	4
34	0
65	1
68	0
36	2
64	1
	16

A: older	
Cat No.	Points
33	3
56	6
59	6
60	0
66	4
67	9
37	5.5
38	1
39	2
61	3
62	4
63	3
	46.5

B: younger	
Cat No.	Points
28	1
29	10
40	5
41	9
42	0
	25

B: older	
Cat No.	Points
27	10
31	8
30	3
	21

Table 13 B

Variability of the Nerve Cells / Age of the Animals

Group	Code	n	$\bar{x}$	s
Controls: younger	C_y	8	0.438	0.904
Controls: older	C_o	8	1.938	2.337
4 ECT's: younger	A_y	11	1.455	1.508
4 ECT's: older	A_o	12	3.875	2.469
11–16 ECT's: younger	B_y	5	5.000	4.528
11–16 ECT's: older	B_o	3	7.000	3.606
	$A_y + B_y$	16	2.563	3.141
	$A_o + B_o$	15	4.500	2.885
$A_{48+96} + B_{48+96}$: younger		7	2.571	3.561
$A_{48+96} + B_{48+96}$: older		6	6.583	2.836

Table 13 C

Variability of the Nerve Cells | Age of the Animals: Differences

Group	C_y	C_o	A_y	A_o	B_y	A_y+B_y	$A_{48+96}+B_{48+96y}$
C_o	1.500 $(0.10 < p < 0.20)$						
A_y	1.017						
A_o		1.937					
B_y	4.562		3.545				
B_o		5.062		3.125			
A_y+B_y	2.125 (p appr. = 0.02)						
A_o+B_o		2.562 $(0.02 < p < 0.05)$				1.937 $(0.05 < p < 0.10)$	
$A_{48+96}+B_{48+96y}$	2.133 $(0.10 < p < 0.20)$						
$A_{48+96}+B_{48+96o}$		4.645 $(p < 0.01)$					4.012 $(0.02 < p < 0.05)$

Table 13 D

Variability of the Nerve Cells / Age of the Animals

Classes	No. of Animals					
	C		A + B		A_{48+96} + B_{48+96}	
	younger	older	younger	older	younger	older
0	6	4	5	1	2	
≦ 1	1		3	1	2	
≦ 2		1	3	1	1	
≦ 3	1			4		1
≦ 4		2	2	2	1	1
≦ 5			1			
≦ 6		1		3		1
≦ 7						
≦ 8				1		1
≦ 9			1	1		1
≦ 10			1	1	1	1

h. COMMENTS

Of the different criteria—*i.e.*, the neuropathological phenomena noted at the third (unbiased) examination—changes in the vessel wall, the glial reaction (including satellitosis) and changes in the nerve cells were found to be those most suitable for differentiating between the animals subjected to ECT and the controls by means of statistical analyses. In a comparison between these criteria relevant to the differences disclosed by them, the changes in the vessel wall and those in the nerve cells may be considered as equally serviceable, and slightly more so than the glial reaction. If the association of the respective phenomena with age is taken into account, the glial reaction appears to lose slightly more in value in comparison to the other two criteria. In such a comparison, the changes in the vessel wall and in the nerve cells still appear to be fairly well matched.

Of the remaining criteria, only pigment in the perivascular spaces and hæmorrhages can be accorded any particular importance, if they are judged on the results of the statistical analyses. In this respect they are comparable. However, in view of the evidently terminal nature of the hæmorrhages, it is extremely doubtful whether this phenomenon is a reliable criterion. Acute cerebral œdema is not serviceable as a basis for differentiating between the respective groups of animals.

If the three most important criteria—*i.e.*, changes in the vessel wall, glial reaction in the parenchyma and changes in the nerve cells—are assembled, the differences between the respective groups could be expected to be accentuated. Such a calculation is shown below. The differences in the scales for evaluating

the various phenomena have, however, been taken into consideration. The values for the vessel wall changes have been divided by 5, in view of the very high mean values for this criterion. The respective totals for each animal, as recorded in Tables 4, 8 A, and 12 were then added together. The mean values and the variances are seen in the following schema:

Groups	n	$\bar{x}$	s^2
C	16	3.43	8.19
A	23	6.53	18.57
B	8	10.91	43.43
A + B	31	7.66	27.56
$A_{48+96} + B_{48+96}$ . . .	13	9.45	40.59

Differences:

C ~ A	$p < 0.02$	($t = 2.70$)
C ~ B	$p < 0.01$	($t = 3.07$)
C ~ A + B	$p = 0.001$	($t = 3.58$)
C ~ $A_{48+96} + B_{48+96}$	$p < 0.01$	($t = 3.15$)
A ~ B	$p < 0.10$	($t = 1.75$)

The differences were thus still further accentuated in this collocation of the criteria, compared to the differences when a single criterion was used. This indicates a relationship between the criteria, *i.e.*, between the neuropathological observations. This must not, however, be interpreted as evidence of an actual relationship. The three phenomena were namely evaluated concurrently, and there is thus a considerable risk that one evaluation might have influenced the next. It nevertheless appears remarkable that the differences were not more noticeable. This indicates that this mutual influence could not have been particularly strong.

Examination of Specimens from Other Parts of the Brain

CHAPTER 12

Changes in the Gyrus Ectosylvius Posterior, Ammon's Horn and the Cerebellum

The findings hitherto reported applied to the gyrus sigmoideus anterior, gyrus sigmoideus posterior and gyrus lateralis. A short account is given in the following of observations made on specimens from the gyrus ectosylvius posterior, Ammon's horn and the cerebellum.

a. GYRUS ECTOSYLVIUS POSTERIOR

The changes observed were, on the whole, of the same nature as those in the three parts of the brain dealt with in previous chapters. They appeared, however, to be somewhat less marked and it was not possible to differentiate, with any degree of certainty, between specimens from the control animals and those subjected to ECT.

b. AMMON'S HORN

It was not feasible, when studying Ammon's horn with its different layers, to set up fixed criteria in the same way as in the case of the other parts of the brain for a differentiation between the controls and the animals that had undergone ECT. This was possibly due in part to the considerable variations in the cytological picture and the not uncommon occurrence of a slight glial reaction—perhaps associated with age—even in the control animals. Moreover, I considered possible neuropathological changes in Ammon's horn to lie somewhat outside the scope of the main object of interest, *i.e.*, the isocortex.

Irreversible lesions (*cf.* Chapter 16) were possibly somewhat more common in Ammon's horn than in the cerebral cortex, but it must be added that phenomena suspect of neuronophagia were also seen in the control animals. The relative cellular polymorphism in some parts of the horn did not facilitate the evaluation. I made no detailed study of Sommer's sector.

c. CEREBELLUM

In the same way as in the case of the cerebral cortex, I tried to set up criteria that would serve to differentiate between specimens from the cerebellum of the control animals and from those subjected to ECT. Among the phenomena sought for, graded and recorded at the third (unbiased) examination were the following: microscopical hæmorrhages, vascular wall reactions, œdema in the lamina dissecans, glial reactions such as "Gliastrauchwerk", and such nerve cell changes as pyknosis, homogeneization (as noted by SPIELMEYER[97]), peripheral vacuolization, decentralization of the nucleus and areas of devastation.

I was not able, on the basis of the presence or absence of the aforementioned phenomena, to differentiate at an unbiased examination between group C and group B. On the contrary, such an attempt resulted in as many of the controls as of the animals subjected to ECT being denoted as shocked. Only one phenomenon, *i.e.*, peripheral (basal) vacuolization in the Purkinje cells, showed a tendency to occur more frequently in the latter category ($0.10 > p > 0.05$). This change consisted of a retraction of the cytoplasm from the cell border, resulting in a small space which was spanned by small trabeculae between the

cytoplasm and the surroundings. In some cases there was a single vacuole, which seemed to push aside the cytoplasm. This phenomenon has been reported by Cerletti & Bini[17], among others. They observed it in the cerebellum of dogs subjected to ECT.

The aforementioned lesion was not, however, so marked that it could be denoted as "stachelige Zellveränderung". It is presumably reversible, since the nucleus is intact. It may be assumed to be caused by ischæmia. This assumption is supported by MORRISON's[74] observation of such peripheral vacuolization—although more pronounced—in experimentally induced ischæmia in dogs and monkeys.

On the whole, however, the cerebellum exhibited scarcely any changes, even in animals subjected to the largest series of ECT's. No hæmorrhages in the parenchyma were found, except in one animal that had undergone ECT. The hæmorrhage was small and of the terminal type—it was thus fresh. Glial reactions in the form of "Gliastrauchwerk"—except possibly of very slight degree—were lacking. No dropping out of cells or other irreversible lesions could be observed.

PART FOUR

THE PRESENT INVESTIGATION: SOURCES OF ERROR AND INTERPRETATION OF THE FINDINGS

CHAPTER 13

Sources of Error Inherent in the Method

a. MANNER OF DEATH, ANESTHESIA AND POST-MORTEM CHANGES

From a theoretical point of view, the manner of sacrificing the animal used in the present investigation, *i.e.*, removal of the brain from the living animal, has both advantages and disadvantages. One obvious advantage is that the time between the "death" of the brain or its disconnexion from the circulation and the placing of the specimen in the fixation medium is considerably shortened. In other words, the inevitable post-mortem changes have a shorter time in which to develop and should thus be decreased in the same proportion. If the animal is sacrificed first—for example, by decapitation—and the brain prepared afterwards, this time is prolonged by approximately 15 minutes, in addition to the time required for removing the specimens and placing them in the fixative.

It is an established fact that post-mortem changes take place very rapidly. KŒNIG[55] showed, in her experiments on guinea-pigs, that changes could be observed as soon as after 30 minutes, mainly in the form of "an early fragmentation of Nissl bodies". This fact must be borne in mind, particularly when it is a matter of estimating changes in the nerve cells; the procedure used in the present investigation therefore appeared to be suitable. But even if the post-mortem changes can thereby be appreciably lessened, they can never be avoided entirely with any method (it is possible that they might have been even slighter with the use of perfusion fixation). It is thus important to standardize the procedure as far as is possible and to have a control material treated in the same way. Reference is made to page 37, on which the method is discussed from this point of view.

One of the disadvantages of the method is that it requires the use of anesthesia. This can, however, be standardized to some extent but it will still

involve a number of slight cerebral changes—with ether narcosis, vascular dilatation in particular. This could be observed in all the specimens from both the control and the treated animals. However, neither the ether anesthesia itself nor the possible cerebral hypoxæmia associated with it can explain the difference between the respective categories with regard to the changes observed. Nor can the nerve cell changes and the glial reactions observed be attributed to these factors. The time elapsing between the induction of anesthesia and fixation was namely too short for such changes to have developed.

On the other hand, when considering the changes in the vessel wall, there is more reason to suspect that they might have had some connexion with the ether narcosis. This question has already been discussed in Chapter 11. I wish, however, to stress the following points. A more or less generalized, passive dilatation of the perivascular spaces was noted in both the control animals and those subjected to ECT. This might possibly be ascribed to the anesthesia and the operation or to the effects of the fixation medium. There is a distinct difference between this phenomenon and the relatively specific phenomenon of sac-like perivascular spaces containing histiocytes found in the small vessels. Moreover, the incidence of the latter phenomenon was found to be appreciably higher among the animals subjected to ECT than among the controls.

Another disadvantage of the method is that the brain is exposed to mechanical intervention—even though of a mild nature—on removal and before fixation. Thus Scharrer[89], for example, pointed out that he could easily induce slight nerve cell changes in the form of "dark cells" by simple mechanical means, such as pressure or traction on the unfixed cerebral tissue. I found such cells in a few of my specimens and denoted this phenomenon as simple shrinkage. The question then arises whether such shrunken nerve cells may be confused with the changes in the nerve cells described in this paper. I consider that it is possible to distinguish between the shrunken nerve cells and the other type since they differ considerably from each other.

In the artefacts, the peripheral parts of the cell in particular appeared to be increased in density, with a concurrent increase in the width of the pericellular spaces, whereas the nucleus was often unaffected. Or, the cell as a whole was strongly shrunken, although more markedly in the periphery, but peripheral chromophobia was lacking. The nerve cell changes with a higher incidence among the animals that had undergone ECT were usually manifested as generally chromophobic cells or, more frequently, as cells with nuclear hyperchromatism and peripheral chromophobia. The use of a specific staining method, such as gallocyanin-chromalum staining, should assist materially in distinguishing between simple shrinkage and nuclear hyperchromatism combined with peripheral chromophobia. A more unspecific method, which often results in marked co-staining, may involve a risk of including both phenomena under the heading of pyknosis. Finally, the focal distribution of

chromophobic cells of Einarson's type 2 and the variability with regard to chromophobia in these areas cannot be explained as shrinkage artefacts alone.

b. FIXATION, SHRINKAGE AND EXTRACTION

The use of chemical fixatives also involves a varying degree of shrinkage and distortion of the cerebral tissue, in addition to other artefacts such as extraction of certain cell components. Various technical procedures have been suggested to eliminate such artefacts. Thus, perfusion fixation with formalin in colloidal solutions of, for example, acacia, has been advised, perhaps mainly to avoid the "dark cells" described by Sharrer and post-mortem changes.

Obviously, still more rapid fixation is obtained with the freezing-drying method[34]. I used this technique parallel with fixation in Carnoy's solution. This is the reason for which the possibility of perfusion fixation did not arise. However, in my opinion, the freezing-drying method is considerably more erratic than chemical methods and thus necessitates discarding a relatively large number of specimens. In addition, only very small pieces of tissue can be used. I therefore used material fixed in Carnoy's solution for the histological examinations.

This fixation method is especially suitable for subsequent staining with gallocyanin-chromalum, since it involves little loss of the nucleic acids, and staining with gallocyanin-chromalum is brought about by selective binding to these substances (*cf.* p. 38). On the other hand, there is a considerable loss of other cell components, mainly lipids, as has been demonstrated by BRATTGÅRD & HYDÉN[11]. In view of the ability of chloroform to dissolve lipoproteins, the method is presumably subject to appreciable sources of error with respect to the pigment content. It is therefore unwarranted to regard the cell that has undergone fixation as a simple coagulation model of the living cell.

It is, however, evident that fixation artefacts are only of any great importance when changes in the nerve cells are concerned, whereas the other phenomena dealt with in the present study—with the exception of the presence of pigment—cannot be thought to be affected by fixation. It is for this reason that changes in the nerve cells were accorded any importance only if they had a characteristic focal distribution with variations in chromophobia within the focus. An attempt to estimate more generalized chromophobia in specimens from animals subjected to ECT proved to be unfeasible in an unbiased examination (*cf.* p. 69). The sources of error in the form of artefacts are still greater in this case and may possibly explain the aforementioned failure. This does not rule out the possibility of the occurrence of generalized chromophobia after ECT, as may well be envisaged on theoretical grounds.

When using chemical methods of fixation, both shrinkage and a loss of cell

components due to extraction must be taken into account. In order to ensure accurate evaluations, it is therefore necessary to resort to a *comparison* between specimens treated as far as possible uniformly, *i.e.*, equivalent pictures, as was earlier pointed out by Nissl. The prerequisites are standardization of the fixation method, an adequate series of controls and statistical analyses of the results. Finally, it is necessary to presuppose a random distribution of the fixation artefacts throughout the material. Or, if this is not the case, the artefacts must bear a correlation to the treatment of which it is desired to demonstrate the effects. In this event, they can scarcely be regarded as artefacts.

c. STAINING

For the reasons given in the aforegoing, it is evident that a progressive staining method is preferable to a regressive method. This is because in the former case the intensity of staining is maximum under standardized conditions, whereas in the latter, differentiation may vary from specimen to specimen, according to a more or less subjective choice. For staining, as for fixation, it is necessary to assume a random distribution of the variations. A subjective factor such as that inherent in a regressive method is always associated with the risk of a systematic error.

The possible contribution of fixation artefacts to the failure to evaluate generalized chromophobia in the specimens from animals that had undergone ECT was mentioned in the previous section. This also applies to the staining method. Certain specimens were less intensely stained; there was thus a variation in the general stainability even under standardized conditions (such as standardization of the pH). In addition to a variation in the binding of the dye, for which it seems justified to assume a random distribution, a variation in the thickness of the sections may be a contributory factor.

d. THICKNESS OF THE SECTIONS

A Reichert sliding microtome was used for sectioning the specimens. Its automatic feeding mechanism was checked with a tachometer and was found to be accurate for practical purposes.

The sections may nevertheless vary in thickness, depending on such factors as the degree of hardness in the different specimens or in different parts of the same specimen, the elasticity of the knife and of the block, the angle at which the knife is directed to the specimen, and the temperature. Because cerebral tissue is soft in consistency, this variation is presumably less than in hard tissue but, according to RICHARDS[83], the embedding process also plays an

essential role. This was also standardized, the same kind of paraffin being used for all the material. All the specimens were sectioned with the same microtome, in the same room and at room temperature and all the preparation was carried out by the same laboratory technician. This does not, however, rule out any variation in the thickness of the sections. It need scarcely be taken into account as a source of error in interpreting the results, since the variation may be assumed to have a random distribution. Strands of vessels and other hard areas were obviously present in all the blocks throughout the material; this also applies to such factors as a lack of homogeneity in the paraffin.

The number of sections was large, as was the number of blocks from each animal; the specimens from the controls and from the treated animals were mixed together—all the specimens from one area of the brain were namely sectioned consecutively before starting on the next area. All these facts rule out the possibility of the variation being a systematic error. The error must instead have been distributed in a very high degree at random.

e. AREA OF THE SECTIONS

Even though efforts were made to cut the specimens of the brain the same size, there must have been an appreciable variation in the area of the individual specimens. For example, the direction of the block in relationship to the knife could vary, although it was tried to keep the conditions uniform in the whole material, *i.e.*, sectioning perpendicularly to the surface of the cortex and transversely to the gyri.

Such a variation in the area of the section must also be envisaged as a source of error in the evaluations, since the interpretation of the properties may be influenced by the size of the specimen. The following investigation was made to ascertain the magnitude of this source of error.

All the specimens of the gyrus sigmoideus anterior (groups C, A and B) studied at the third examination were projected in a photographic magnifying apparatus at constant magnification onto the same sort of photographic paper, and the outlines drawn in by the laboratory technician. These enlargements were then cut out and weighed separately by another technician. With a linear magnification of 3.65 ×, corresponding to a surface magnification of 13.32 × (no correction was made for the optical distortion on magnification, since it could obviously be neglected) and a paper weight, in which one mg corresponded to 9.410 mm^2, the area of the corresponding sections could easily be computed.

The specimens from groups C, A and B diagnosed as S or (S) at the third (unbiased) examination were set in relation to those diagnosed as 0, (0) or [(0)]. The agreement in the size of the area was found to be extremely good, as is seen from the following figures.

Group	n	$\bar{x}$ mm²	s mm²
S + (S)	26	39.75	12.72*
0 + (0) + [(0)] . .	68	38.92	

The difference between the two groups amounted to 0.83 mm², $t = 0.29$ and $0.7 < p < 0.8$.

The error of the method was obtained by taking two sections from each specimen of the brain; double values were thus recorded in each case. The error of the method, calculated as the standard deviation, amounted to 1.52 mm².

The following conclusion may therefore be drawn. The difference between the area of the sections in the two groups was not of such an order of magnitude that it could have been of any importance as a source of error in the main investigation.

Summary

The sources of error and artefacts of a technical nature—*i.e.*, those inherent in the experimental conditions—discussed in the aforegoing can be reduced to a considerable extent by standardization of the technique. They cannot, however, be eliminated entirely. In the present investigation, they were of a markedly random nature; their random distribution was accentuated by the arrangement of the material, *i.e.*, mingling the specimens from the control animals with those from the animals subjected to ECT. Statistical analyses of the data obtained should permit them to be ruled out as causative factors of the differences observed between the respective groups.

CHAPTER 14

Sources of Error due to the Choice of the Material

a. AGE DISTRIBUTION

The age distribution of the control animals and of those subjected to ECT has already been discussed in Chapter 4. It was then found that there was no difference in this respect between the two categories of animals.

* This value was calculated on a class variation with a class interval of 3.53 mm, corresponding to 5 mg of the paper.

b. CHANGES OF ENCEPHALITIC TYPE

At the various examinations, small round-cell infiltrations (infiltrations or accumulations of mononuclear cells) beside the blood vessels were observed in a few animals, both controls and those that had undergone ECT (see Figs. 8 och 9). No large, independent granulomas were found. These accumulations of round cells were nevertheless so characteristic that they could be distinguished from the perivascular changes presumably associated with ECT. They indicated the existence of some form of inflammatory process, so-called encephalitic changes.

That they could be distinguished from the neuropathological phenomena described in earlier chapters is evident from the fact that they were observed in three controls (Cats nos. 20, 49 and 52): at the unbiased examination these animals were diagnosed as not shocked. The incidence is shown in Table 14.

Table 14

Cats with Encephalitic Changes

Cat No.	Group	Diagnosis at 3rd Exam.				Degree of Encephalit. Changes
		ant.	post.	lat.	total	
20	C	E	E	(S)	Not shocked	marked
49	»	(E)	0	0	»	very slight
52	»	S	0	0	»	slight
55	A_{21}	E	0	0	»	marked
57	»	0	0	0	»	very slight
60	»	E	E	E	»	marked
68	A_{48}	E	(S)	0	»	slight
39	A_{8d}	0	0	0	»	very slight
64	»	(E)	0	S	»	very slight

Moreover, none of the animals subjected to ECT and exhibiting these round-cell infiltrations were diagnosed as shocked. This indicates that I was possibly still more cautious in making a positive diagnosis when signs of so-called encephalitic changes were present. I wish once more to emphasize that there was a distinct difference between these phenomena and those described earlier under the heading "Changes in the Vessel Wall".

*

It may be inferred from the results reported that there were individual variations with regard to the sensitivity to ECT. No satisfactory analysis could be made of this individual variation, but it may be noted that age appeared to play some rôle. Without a control series and statistical analyses, there are

thus considerable risks of erroneous conclusions. On practical grounds it is, however, necessary to confine the material to a limited number of animals, and an examination of the entire brain is scarcely feasible even then.

c. SELECTION OF THE SPECIMENS

Seen under the microscope, the brain offers practically unlimited scope for study. The topography varies from one part to the next and even, to a great extent, from one section to another and from visual field to visual field. From a practical viewpoint, it is impossible to examine the entire brain unless such an extensive method is chosen—for example, a macroscopical evaluation—that the details are lost to view. Conversely, the more intense the study of the details, the greater is the necessity—on practical grounds—of limiting the extent of the samples.

Every worker embarking on a study of neuropathological phenomena of this type is therefore faced with the question: *how extensive or intensive is the examination to be?* Obviously, there is a possibility of combining extensive and intensive methods: for example, a macroscopical study complemented by a microscopical examination of the parts considered to be suspect in some way at the former examination. Such a procedure is not above criticism. This is because a subjective selection on the basis of criteria varying from one case to the next involves risks when drawing conclusions with regard to the material as a whole.

Another method is available which is more suitable for comparisons between animals treated in different ways. This is to take specimens from the brain of all the animals, under conditions as uniform as possible, particularly specimens of the same size and localization. But, even if this procedure is less open to criticism as a method of selection, it nevertheless implies a selection. The question of the *representativeness* of the material then arises. If the changes to be studied are uniformly distributed throughout the brain the matter is less controversial, but we have usually little advance knowledge of the conditions.

In the present investigation, I was obliged to assume that even if the distribution of the cerebral changes accompanying ECT was not homogeneous throughout the brain, it was presumably fairly uniform in the respective parts of the cortex. There was thus reason to anticipate a relatively accurate picture if not too small specimens were taken from different parts of the brain. The finer focal distribution of the altered nerve cells need scarcely be taken into consideration in this connexion.

Fig. 3, showing the parts of the brain selected, and the account given on page 37 are put forward as evidence that the choice of specimens was not incorrect in view of the problem. Attention is drawn to the topography in

relationship to the line drawn between the centres of the electrodes. The gyrus sigmoideus anterior and posterior are not in its immediate vicinity but are considerably nearer to it than the gyrus lateralis. This, in turn, is somewhat closer than the gyrus ectosylvius. There was no appreciable difference between the size of the specimens from the animals in the various groups (*cf.* p. 82).

On the aforementioned grounds, it is presumably justified to consider the parts selected as relatively representative of the cerebral cortex and as suitable for mutual comparisons.

As mentioned earlier, it is necessary—for practical reasons—to choose relatively small parts of the brain for histological examination, *i.e.*, to confine the intensive study to small areas. It would appear desirable to follow this principle further and to select histological details, in particular the nerve cells, for detailed cytological study. However, we are once more faced with intricate problems.

The histological examination revealed that the altered nerve cells had a tendency to a finer focal distribution. Moreover, even in the normal brain, the nerve cells exhibit great cytoarchitectural variations from one part of the brain to the next, from one layer to the next and from section to section. But this variability is by no means random; it follows a relatively fixed cytoarchitecture. It is thus an organization with an extremely widely branched system. This precludes the use of a simple process of random selection for obtaining a representative random sample in the same way as, for example, in the case of a smear of blood. In addition, the cells constitute so small a part of the visual field—which consists mainly of interstitial tissue—that the application of, for example, CHALKLEY's[19] method* is too time-consuming.

A more suitable method of random selection applicable to the nerve cells of the cerebral cortex is therefore a *desideratum*, since extremely refined techniques are now available for a study of their changes. I refer to CASPERSSON's[15, 16] ultra-violet methods: both the indirect method with photography in the different parts of the ultra-violet spectrum—particularly at 2600 Å, at which the nucleic acids have a high specific absorption—and direct quantitative spectrophotometry. These have proved to be eminently suitable for the study of nerve cells. In the present connexion, they may be regarded as extremely intensive methods. Thus, they provide quantitative data, whereas the usual histological techniques with staining only provide more or less satisfactory pictures and the differences between them can only be estimated.

In neuropathological investigations of the type reported here it is, however, necessary for practical reasons to restrict the study to a microscopical evaluation of the different sections. This widens the scope in such a way that the variations in the details are evened out. At present, a subjective estimation still appears to be the best method for a study of experimental material composed of such a complex of details. The object of the present investigation was to determine whether there was a difference between sections of the brain from animals treated in different ways, irrespective of whether these differences were present in or beside the blood vessels, in the glial tissue or in the nerve cells.

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* Chalkley's method consists of adjusting the visual field in such a way that a hair, attached to the diaphragm of the microscope, points to a certain detail—*e.g.*, a certain cell. The details at which similar hairs point in the objective are then ascertained. This permits statistical calculations to be made of the incidence in the section of such elements as cell nuclei, zones of cytoplasm, interstices and blood vessels.

Obviously it would have been preferable to replace the subjective estimations by more objective numerical data—for example, measurements in the ultra-violet range of the spectrum as regards the nerve cells, or a computation of the mean number of satellite cells in each section. This is scarcely practicable on a large scale and such a detailed study would have been too time-consuming even on such a relatively small series as the present one. It did not appear possible to supplant trained subjective estimation by any objective method that would have been equally comprehensive, rapid and effective.

d. SUBJECTIVE FACTORS

GILDEA & COBB[35] studied the cerebral changes in 32 cats with acute cerebral anæmia induced by ligating the blood supply to the brain. They used five cats as controls. The neuropathological changes were, on the whole, considerably more marked than those found after ECT. Despite this fact, the authors stressed the difficulties of making objective observations, in the following memorable words:

"The subjective element is surprisingly great. It is all too easy to look through a microscope and focus one's attention on the few abnormal cells in a normal control animal. To keep the normal constantly in mind and to satisfy ourselves of our accuracy it was frequently necessary to test each other with unknown abnormal and normal slides. The gradations between "normal" and "ischemic" cortex are many and subtle. Only the striking lesions, such as the areas of devastation, could be quickly and surely recognized. Consequently, in the study of such disturbances as these a more specific and refined method must be developed if one is to obtain profitable histologic observations."

I am in complete agreement with this attitude towards the problem. The critical considerations of the aforementioned authors prompted me to use a larger series of control animals and to record my observations on special forms.

These measures did not suffice to eliminate the subjective errors. I do not, however, regret having repeated my use of the technique applied by earlier workers at my second histological examination. This is because at the third (unbiased) examination it provided me—literally speaking—with tangible proof of the intricacy of the task with which one is faced. This was all the more instructive since I believed that I was already unbiased at the second examination. Thus, adequate control material is, in itself, no guarantee of an unbiased evaluation.

The phenomenon of reactive changes in the vessel wall may be taken as an example of these difficulties and a comparison made between its evaluation at the second (biased) and at the third (unbiased) examination.

This example was chosen for the following reason. Judging by the microscopical studies and the results of diagnosing the specimens, this phenomenon seemed to be the most conspicuous and a suitable criterion for differentiating

between the control animals and those subjected to ECT. Compared with the other changes observed, it appeared to imply the lowest risks of faulty evaluation on subjective grounds. When discussing the chromaffinity of the nerve cells, it was emphasized that this feature, since it was more subtle, was more likely to imply an increased risk of bias (*cf.* Chapter 11).

The third examination of the specimens from the gyrus sigmoideus anterior (groups C, A and B, respectively) revealed the following. At the previous examinations (when I was aware of the origin of the specimens) I had definitely tended to underrate this phenomenon in the brain of the control animals. The specimens from the animals subjected to ECT were, on the contrary, estimated fairly uniformly at both the biased and the unbiased examinations. In my opinion, this may be interpreted as an indication that my efforts to be unbiased already at the second examination were not altogether fruitless. But, the error—the evidently unavoidable subjective error—emerged elsewhere, *i.e.*, in the evaluation of the specimens from the controls. My knowledge of their origin apparently suggested me to overlook what I subsequently saw when I did not know that the specimens derived from the control animals. Table 15 illustrates the conditions as adequately as would any further commentary.

Table 15

Changes in the Vessel Wall in the Gyrus Sigmoideus Anterior: Comparison between Results of the Second Examination (not unbiased) and the Third Examination (unbiased)

Classes	No. of Specimens			
	C = Controls		A + B = Treated Animals	
	Exam. III	Exam. II	Exam. III	Exam. II
0	10	22	5	18
1	9	8	8	4
2	12		37	15
3			10	24
n	31	30	60	61
$\bar{x}$	1.065	0.267	1.867	1.738

Differences:

$$C_{III} \sim C_{II}: \frac{19}{12} \sim \frac{30}{0} \quad \ldots \quad p < 0.001 \text{ (exact method: 0.0001)}$$

or

$$C_{III} \sim C_{II}: \frac{10}{21} \sim \frac{22}{8} \quad \ldots \quad p < 0.001 \ (\chi^2 = 17.47)$$

The figures recorded thus show that I had underestimated the changes present in the brain of the control animals when I was aware of the origin of the specimens.

*

It must once more be stressed that, even at the unbiased examination, it was purely a matter of subjective observations made on looking through the microscope. Only with regard to the presence or absence of hæmorrhages were they of a simple, qualitative nature. Otherwise, it was mainly a question of more or less subtle, quantitative grading of phenomena present in varying degrees of saliency in specimens from both the control animals and those subjected to ECT. These details were finally combined in an equally subjective evaluation—the diagnosis.

The actual observations presumably possess a considerable degree of accuracy as compared with their evaluation. Moreover, it is the latter procedure that may most easily be suspected to be biased. It has already been pointed out that it is possible by a simple means—an evaluation without knowledge of the origin of the specimens—to exclude an obviously appreciable source of error, *i.e.*, the prejudiced opinion of the examiner.

One risk is, however, present—namely that the examiner may recognize the specimens. This risk may obviously be eliminated by examining only objects that have never been seen earlier. In the present study the following procedure was used. The third, unbiased examination dealt with new sections, that had been stained and mounted in a special series and had thus not been seen before. This fact, combined with the large number of the specimens—altogether several hundred—should have diminished the risk of recognition to the point of negligibility.

Even if the subjective evaluation does not therefore appear to imply any systematic error of consequence, the random errors must be appreciable. The personal scale of values fluctuates more or less momentarily and possibly still more at longer intervals, training and fatigue being important factors (*cf.* p. 99). Thorough shuffling of the specimens, *i.e.*, a random selection with regard to time, should decrease the effect of this fluctuation. In the present study, the fluctuations in the scale of values were further evened out by the fact that several specimens—as a rule six—from each animal were examined on different occasions and were then mixed with other specimens. The time elapsing between the study of the individual specimens from the same animal amounted to several weeks. This should have levelled out the fluctuations spread over a longer period as well.

Another possibility exists of overcoming the random errors. This is to study the probability of the differences noted—for example, between the control animals and those subjected to ECT—occurring only on the grounds

of random factors. In other words, a statistical analysis may be made of the material.

The p values given in the present study thus denote the theoretical possibility of the respective differences being caused by random factors.

*

The following conclusion may therefore be drawn. In such delicate evaluations as those involved in a neuropathological study of slight, diffuse cerebral abnormalities, the risk of subjectively influenced—and thus scientifically inaccurate—observations appears to be considerable. Gildea & Cobb, quoted earlier, felt strongly on this matter and advocated the development of a more specific and refined method. Their views are unquestionably shared by many other neuropathologists. It was pointed out in the survey of the literature that there were considerable differences in the observations made by various workers; this could not be satisfactorily explained on the grounds of the differences in the experimental techniques. In my opinion, such subjective factors as those discussed may very well be the reason for these discrepancies.

CHAPTER 15

The Control Examination

Motive

Because the observations made proved to be difficult to estimate and therefore hard to check, it was considered expedient to submit the results to a control examination. This was also intended to test the reliability of the method.

a. SCOPE AND PLANNING

On practical grounds, it was necessary to limit the control examination to the smallest number of specimens that could be expected, from the theoretical point of view, to give unambiguous results. It was therefore confined to 8 control animals and 8 subjected to ECT and diagnosed at the third examination as shocked. Their distribution is shown in Table 16. Six blocks with specimens from the right and left gyrus sigmoideus anterior, the right and left gyrus sigmoideus posterior and the right and left gyrus lateralis were taken from each of these animals, *i.e.*, altogether 96 specimens.

The specimens, together with a list of their numbers, were handed over to an impartial pathologist*. The specimens were numbered afresh by him; the

* Dr. Fredrik Wahlgren, Hon. M.D., of Södersjukhuset, Stockholm.

Table 16

List of Experimental Animals: Control Examination

No. Allotted by:			
Hartelius	Wahlgren	Hartelius	Wahlgren
Controls		Animals Subjected to ECT	
44	13	56	14
46	27	66	16
47	39	67	32
48	17	37	38
49	24	29	41
50	52	27	19
51	22	31	6
53	18	30	3

numbers showed which specimens came from the individual animals. (Each number consisted of a figure and a letter, *e.g.* 32a, 32b, a denoting the *right* gyrus sigmoideus anterior and b the *left* gyrus sigmoideus anterior.) On the other hand, I was not informed of the relationship of the new numbers to the old ones, *i.e.*, which numbers had been allotted to the animals subjected to ECT and which to the controls. The lists of the old and new numbers were retained by the pathologist in question and the specimens with their new numbers were returned to me.

My task was then to determine—in the same way as at the third examination—by means of a histological study which of the series of specimens had been taken from animals subjected to ECT and which from control animals.

b. PROCEDURE AND RESULTS

The same histological technique as that used earlier was applied in this examination (denoted hereafter as the fourth examination), *i.e.*, sections 5 μ and 10 μ thick and staining with gallocyanin-chromalum and van Gieson's method. For the differential diagnosis between shocked and not shocked specimens I used gallocyanin-stained specimens only, mainly 5 μ thick. Sections 10 μ thick were useful as complementary material. I found staining with van Gieson's method to be of little help.

1. *The Diagnoses*

Protocols were drawn up in the same way as earlier. After completing the examination, the following list was sent to the pathologist. He then supplied me with the list—which I had not seen before—of the new numbers allotted by him to the animals.

Diagnoses

Results of the Control Examination

Animals diagnosed as controls: Nos. 17, 18, 22, 24, 27, 39, 52
No. 27 denoted as doubtful.

Animals diagnosed as subjected to ECT: Nos. 3, 6, 14, 16, 32, 38, 41
Nos. 3, 38 and 41 denoted as doubtful.

With regard to Cat no. 19, I observed definite "encephalitic" changes which makes it more difficult to decide whether or not "shock" phenomena were also present. In addition to the "encephalitic" changes (round-cell infiltrations beside the blood vessels) there seemed to be changes of the type I thought I had observed after ECT. I may, however, have confused these with the "encephalitic" changes, although this appears less probable.

I am most doubtful with regard to Cat no. 13; I consider that either this animal or no. 19 is a control. The former animal seems, however, to be the most likely one.*

The problem was thus the following. The material consisted of sections from the brain of 8 control animals and 8 animals subjected to ECT: the animals were to be assigned to the respective categories by means of a histological study of the specimens. On the hypothesis that the method used was entirely lacking in precision—implying that the animals were actually selected purely at random—the probability of classifying the animals correctly was less than 0.0001. Such a low degree of probability is a strong argument in favour of the accuracy of the method. The diagnosis was doubtful in two cases. If these cases are not included in the calculation, the corresponding probability is approximately 0.0003; the aforegoing statement is therefore valid.

The conclusion drawn from the results of the third examination regarding the possibility of diagnosing cerebral changes produced by ECT thus appears to be confirmed by this (the fourth) examination.

A comparison may also be made between the protocols for the two unbiased (third and fourth) examinations with respect to the special neuropathological observations recorded. It must, however, be borne in mind that although the same parts of the brain were examined on both occasions, the sections were not the same ones. Moreover, there was a long interval—several months—between the two examinations. There is therefore no reason to anticipate a similarity in every detail. The results reported in the following nevertheless show the degree of conformity pertaining to such phenomena as changes in the vessel wall, glial reactions and changes in the nerve cells.

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* English translation of the original list sent to Dr. Wahlgren.

The changes were evaluated in the same way as at the third examination, *i.e.*, using a four-grade scale: 0, 1, 2 and 3 (and occasionally half-grades). The six specimens thus resulted in a total for the individual animal that could vary between 0 and 18. The totals obtained in the two examinations are recorded in Table 17 A.

Table 17 A

Comparison between Results of Third and Fourth Examinations: Changes in the Vessel Wall

Controls		
Cat No.	Points Exam. III	Points Exam. IV
44	5	12
48	12	6
53	3	4
51	8	6
49	4	1.5
46	7	6
47	6	1
50	8	2.5
	53	39

Treated Animals		
Cat No.	Points Exam. III	Points Exam. IV
30	13	6
31	12	12.5
56	10	10
66	16	11.5
27	18	17
67	18	13.5
37	11	10.5
29	16	7
	114	88

It is evident from the table that there were considerable differences in the individual values at the respective examinations. It may also be inferred that at the fourth examination the scale was, on the whole, higher (*i.e.*, with lower values) than at the third, whereas there is good agreement between the mean values for the ratio of the control animals to those subjected to ECT.

	III*	IV*
$\frac{C}{ECT}$	$\frac{53}{114} = 0.465$	$\frac{39}{88} = 0.443$

A *t* analysis gives the following results.

$C_{III} \sim ECT_{III}$ . . . . . $p < 0.001$ $(t = 5.09)$

$C_{IV} \sim ECT_{IV}$ . . . . . $p < 0.01$ $(t = 3.47)$

The difference between the control animals and those subjected to ECT with respect to the changes in the vessel wall that could be noted at the third examination was thus confirmed at the fourth examination.

* III denotes the third examination, IV the fourth examination.

3. *Glial Reaction in the Parenchyma*

Table 17 B shows a comparison between the results of the third and fourth examinations with respect to the glial reaction; the basis is the same as for the changes in the vessel wall. A three-grade scale was used: 0, 1 and 2 (and

Table 17 B

Comparison between Results of Third and Fourth Examinations: Glial Reaction in the Parenchyma

Controls			Treated Animals		
Cat No.	Points Exam. III	Points Exam. IV	Cat No.	Points Exam. III	Points Exam. IV
44	0	2	30	3	2.5
48	0	1	31	3	1.5
53	0	0	56	4	3.5
51	0.5	0	66	3	3.5
49	0	0	27	4.5	3.5
46	0.5	0.5	67	7	4
47	0.5	0	37	3	2
50	0.5	0	29	5	1.5
	2.0	3.5		32.5	22

occasionally half-grades)—*cf.* Chapter 11. Grade 2 was, however, very infrequent. Even a cursory glance at the table discloses good agreement between the results of the two examinations. A t analysis gave the following figures.

$$C_{III} \sim ECT_{III} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad p < 0.001 \ (t = 5.29)$$
$$C_{IV} \sim ECT_{IV} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad p < 0.001 \ (t = 7.45)$$

It is true that there is a considerable skewness in the distribution in both C_{III} and C_{IV}, but the result nevertheless appears convincing. This also applies to Table 17 C showing the class variation. The observations made at the fourth examination in respect of the glial reaction were thus in good conformity with those made at the third examination.

4. *Satellitosis*

In both the third and fourth examinations, satellitosis was recorded according to a four-grade scale: 0, 1, 2 and 3 (and half-grades). The following figures show the comparative results of the respective examinations relevant to the evaluation of satellitosis. It is seen that the scale was somewhat higher (*i.e.*, the values were lower) at the fourth examination than at the third, but that the respective ratios of the control animals to those subjected to ECT were in fairly good agreement. (*Cf.* Table 17 D).

Table 17 C

Comparison between Results of Third and Fourth Examinations: Glial Reaction in the Parenchyma

Classes	No. of Animals			
	Exam. III		Exam. IV	
	C	ECT	C	ECT
0	4		5	
≤ 1	4		2	
≤ 2			1	3
≤ 3		4		1
≤ 4		1		4
≤ 5		2		
≤ 6				
≤ 7		1		
≤ 12				

Differences:

$C_{III} \sim ECT_{III}$: $\frac{8}{0} \sim \frac{0}{8}$ . . . . . $p = 0.0001$

$C_{IV} \sim ECT_{IV}$: $\frac{7}{1} \sim \frac{0}{8}$ . . . . . $p = 0.0007$

$$\frac{C_{III}}{ECT_{III}} = \frac{66}{111} = 0.59$$

$$\frac{C_{IV}}{ECT_{IV}} = \frac{39}{81} = 0.48$$

A t analysis gave the following figures.

$C_{III} \sim ECT_{III}$ . . . . . $p < 0.001$ ($t = 5.17$)

$C_{IV} \sim ECT_{IV}$ . . . . . $p < 0.001$ ($t = 4.86$)

Table 17 D

Comparison between Results of Third and Fourth Examinations: Satellitosis

Controls		
Cat No.	Points Exam. III	Points Exam. IV
44	10	10
48	12	4
53	5	3
51	11	6
49	6	4
46	7	6
47	5	2
50	10	4
	66	39

Treated Animals		
Cat No.	Points Exam. III	Points Exam. IV
30	13	8
31	12	11.5
56	14	10
66	13	10
27	14	11.5
67	15	13
37	14	9
29	16	8
	111	81

A class variation table (Table 17 E) illustrates the aforementioned displacement in the scale.

The t analysis permits the following inference to be drawn with respect to the phenomenon of satellitosis, as it was evaluated in the present study. The results of the control examination were in conformity with those of the third examination as far as a difference between the two categories of animals is concerned.

Table 17 E

Comparison between Results of Third and Fourth Examinations: Satellitosis

Classes	No. of Animals			
	Exam. III		Exam. IV	
	C	ECT	C	ECT
0				
≦ 3			2	
≦ 6	3		5	
≦ 9	1			3
≦ 12	4	1	1	4
≦ 15		6		1
≦ 18		1		

Differences:

$C_{III} \sim ECT_{III}$: $\frac{8}{0} \sim \frac{1}{7}$ . . . . . $p = 0.0007$

$C_{IV} \sim ECT_{IV}$: $\frac{7}{1} \sim \frac{0}{8}$ . . . . . $p = 0.0007$

5. *Hæmorrhages*

Small, usually petechial, hæmorrhages (small extravasations) of the fresh type were observed at both the third examination and the fourth. They were entirely lacking in some of the six specimens from each animal. Table 17 F shows the number of specimens out of the six examined in which such fresh (terminal) hæmorrhages were present. The total value allotted to each animal thus varied between 0 and 6. Obviously, this is only a rough estimation of the incidence of hæmorrhages, which is subject to considerable sources of error, such as variations in the surface area of the section. The results are nevertheless recorded for two reasons. Firstly, they illustrate the similarity in the results of both examinations. Secondly, the difference between the control animals and those subjected to ECT is disclosed by both examinations.

The following figures were found on a t analysis.

$C_{III} \sim ECT_{III}$ . . . . . $0.01 < p < 0.02$ $(t = 2.73)$

$C_{IV} \sim ECT_{IV}$ . . . . . $p < 0.01$ $(t = 3.26)$

Table 17 F

Comparison between Results of Third and Fourth Examinations: Hæmorrhages

Controls		
Cat No.	Points Exam. III	Points Exam. IV
44	1	2
48	1	1
53	0	1
51	4	3
49	0	0
46	0	1
47	0	1
50	1	1
	7	10

Treated Animals		
Cat No.	Points Exam. III	Points Exam. IV
30	2	3
31	4	4
56	4	4
66	4	3
27	2	1
67	4	6
37	1	3
29	1	2
	22	26

The ratio between the mean values is also in agreement.

$$\frac{C_{III}}{ECT_{III}} = \frac{7}{22} = 0.32$$

$$\frac{C_{IV}}{ECT_{IV}} = \frac{10}{26} = 0.38$$

Table 17 G shows the class variation and a similar distribution in the third and the fourth examinations.

Table 17 G

Comparison between Results of Third and Fourth Examinations: Hæmorrhages

Classes	No. of Animals			
	Exam. III		Exam. IV	
	C	ECT	C	ECT
0	4		1	
1	3	2	5	1
2		2	1	1
3			1	3
4	1	4		2
5				
6				1

Differences:

$$\left.\begin{array}{l} C_{III} \sim ECT_{III}: \ \frac{7}{1} \sim \frac{2}{6} \\ C_{IV} \sim ECT_{IV}: \ \frac{6}{2} \sim \frac{1}{7} \end{array}\right\} \quad \ldots\ldots\ldots \quad p < 0.05 \text{ (Mainland: } 0.0203 \times 2)$$

6. *Changes in the Nerve Cells*

It was pointed out in Chapter 11 that the most distinct change in the nerve cells was an increased variability in the chromaffinity and that the focal arrangement of this phenomenon was striking. At the third examination, this variability was evaluated on a three-grade scale: 0, 1 and 2. In the expectation of obtaining more emphatic data, I used a four-grade scale at the fourth examination: 0: no variability; 1: slight variability; 2: some variability and 3: marked variability. It may be deduced from Table 17 H that my expectations

Table 17 H

Comparison between Results of Third and Fourth Examinations: Variability of the Nerve Cells

Controls		
Cat No.	Points Exam. III*	Points Exam. IV*
44	0	12
48	2.5	5.5
53	0	1
51	0	8
49	0	3
46	0	6
47	0	2
50	0	4.5
	2.5	42

Treated Animals		
Cat No.	Points Exam. III*	Points Exam. IV*
30	3	5.5
31	8	12
56	6	12
66	4	14
27	10	18
67	9	16
37	5.5	8.5
29	10	7
	55.5	93

* Note: Different scales.

were not fulfilled to any great extent. A comparison between the results of the third and the fourth examination, respectively, nevertheless discloses a relatively good agreement in the differences between the two categories of animals with respect to this phenomenon.

A t analysis resulted in the following figures.

$$C_{III} \sim ECT_{III} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad p < 0.001 \ (t = 6.6)$$

$$C_{IV} \sim ECT_{IV} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad p < 0.01 \ \ (t = 3.20)$$

The skewness was so striking in the third examination that a t analysis scarcely appeared to be warranted. The figures in the table nevertheless afford convincing evidence of the difference between the controls and the treated animals. At the fourth examination, the distribution appeared to justify a t analysis.

The following conclusion may therefore be drawn. The dissimilarity in the scale of evaluation at the third and fourth examination, respectively, was

intentional. At the former examination, a difference was noted between the controls and the treated animals with regard to the changes in the nerve cells. The aforementioned dissimilarity did not prevent the appearance of this difference at the fourth examination as well.

C. COMMENTS

A comparison was made between the results of the two unbiased examinations, *i.e.*, the third and the fourth (control) examination, respectively. It disclosed a conformity in the results, with respect both to the diagnosis of the animals and to that of the individual pathological phenomena: changes in the vessel wall, glial reactions, satellitosis, hæmorrhages and changes in the nerve cells. This fact obviously corroborates the conclusions drawn from the third examination.

An interesting feature is that the scale used to evaluate the individual phenomena fluctuated from one examination to the next, but the fluctuation was not the same for the different phenomena. The agreement is best in the case of the most unambiguous changes, *i.e.*, hæmorrhages. With this exception, the values were consistently lower at the fourth examination, indicating that the grades were higher. Despite this fact, the ratios between the mean values for the controls and the animals subjected to ECT were in strikingly good agreement.

The following conclusion may therefore be drawn. It is possible to repeat an unbiased examination, but only as a comparison between controls and treated animals. In other words, a comparison must be made simultaneously; a lapse of time between two estimations of the same material may distort the results owing to a change in the examiner's scale of values.

In my opinion, it is not feasible—in an examination of such phenomena as those in question—to rely upon a mental recollection of control material seen at an earlier date. Nor does it appear reasonable to study all the specimens from one category first and then those from the other. The only possibility of eliminating the sources of error due to the subjective element is an unbiased examination of both categories (controls and treated animals) concurrently—*i.e.*, distributed at random with respect to the time factor.

CHAPTER 16

Possibly Irreversible Changes

It may be inferred from the description of the neuropathological phenomena given in Part Three that they were mainly of a mild nature and that the majority were presumably reversible.

When making a closer analysis of the question of irreversibility, the length of the survival time appears to be of prime importance. This is because the changes observed were found to be most distinct in the animals that were allowed to survive the last ECT for 48 to 96 hours, whereas they were less marked in those with a shorter or longer survival time, respectively. This indicates that the changes in question are associated with the ECT, but that they require a certain time to develop, after which they become maximum and subsequently recede. This latency time, in turn, indicates that the changes are presumably associated with a reparative phase. On the other hand, they can scarcely constitute the morphological correlate of the transient cerebral dysfunction of short duration that can be recorded in the form of transitory loss of consciousness, and the subsequent decrease in mental acuity of the animal immediately after the seizure.

With regard to the changes in the vessel wall and the glial reaction, not only this time factor but also the nature of the changes argue in favour of their reparative character. To the extent that they are of a reparative nature, they may also be assumed to be largely reversible. The disturbances in the blood-brain barrier and in the permeability of the vessel wall suggested by the changes noted are presumably reversible within the same short period as the slight cerebral œdema to which they may give rise. A phenomenon which might be of longer duration would be a decreased permeability associated with repair. However, on the grounds of the great adaptability of the vascular tissue, this is presumably an inconsiderable risk.

The glial changes—of a productive nature—may also be presumed to be mainly reversible, since they chiefly affect the most reactive part of the glial tissue (the microglia and possibly the oligodendroglia). A possible slight, more or less permanent, increase in number of certain glial elements would scarcely disturb the function of the cerebral parenchyma.

Of more importance in this connexion is the nature of the nerve cell changes. The perivascular occurrence of pigment of the autogenic type may appear more ominous. Obviously, no pathological importance connected with the ECT need be accorded to the intracellular presence of such pigment. But when it is phagocytozed or lies in the perivascular spaces, it may be interpreted as an indirect sign of disintegration of the nerve cells. With regard to this phenomenon as well, a peak was noted in the animals subjected to ECT with a survival time of medium length. This appears to afford fairly strong but nevertheless indirect evidence that the ECT may produce disintegration of the nerve cells. More direct indications are, however, necessary before the question of the occurrence of irreversible changes can be answered conclusively.

In the present material, the nerve cell changes noted were slight and fairly infrequent. The majority of the nerve cells, even in those animals subjected to most intense treatment, exhibited no changes. Those observed could not be

denoted, without stronger evidence, as irreversible. The prognosis for the individual cell must be made on the basis of an instantaneous picture (more or less distorted by artefacts) of a particular process, of which the final outcome may be either subsequent satisfactory function or death, or possibly some intermediate condition. It is therefore necessary to establish criteria of reversibility and irreversibility.

When setting up such criteria, it is useful to study the sequelæ of other injuries in which the changes are more conspicuous than those in ECT. In view of the conclusions drawn from the considerations on the nature of the nerve cell changes in the present connexion (*cf.* Chapter 17) it appears suitable to use as a basis the cytological changes observed in experimentally induced ischæmia.

The literature on such experiments, both on the spinal cord and on the brain, is extensive. Mention may be made of the investigations of KROGH[57] and of HOCHBERG & HYDÉN[44]; HÄGGQUIST's[49] method for inducing ischæmia in the spinal cord of rabbits was used in both cases. Krogh used the gallocyanin-chromalum staining method. He found that, with a mild degree of ischæmia, the nerve cells rapidly returned to normal. With more severe damage, they progressed *via* acute swelling, Nissl's profound cell changes, Spielmeyer's ischæmic cell changes, and/or pseudosclerosis to shadow cells, whereafter they rapidly disappeared. The final stage was thus a shadow cell, which must be regarded as a definitely irreparable stage.

Hochberg & Hydén[44] used Caspersson's technique with absorption measurements in the ultra-violet range of the spectrum. Their interest was focused mainly on the mechanisms of restitution after the ischæmic injury. They stressed that the disappearance of the nucleic acids and proteins in the nucleolus is a severe prognostic sign. The final stage was thus disorganization of the nucleolar apparatus and subsequent neuronophagia.

Gildea & Cobb[35] studied the nerve cell changes in experimentally induced cerebral anæmia in the cat. They stated that the most severe lesions, *i.e.*, areas of devastation, were only found in those animals that had survived for at least 24 hours. The disintegration of the nerve cells was preceded by a shadow cell stage.

In similar experiments, GRENELL[38] noted that neuronophagia was frequently the final stage in the irreversible nerve cell changes. He also stressed that the isocortex is most susceptible to ischæmia but that, on the whole, the lesions were relatively diffusely spread throughout the brain, a fact also pointed out by Gildea & Cobb[35].

Scholz pointed out that in brain damage caused by epilepsy, the ischæmic ganglion cell necrosis is not visible until 15—24 hours after the seizure (*cf.* Krogh[57]). It can subsequently be observed for a few days only, after which time the cells disintegrate, leaving practically no traces. Obviously, there are

considerably greater possibilities of noting changes in the nerve cells than of discerning a more or less diffuse—elective—dropping out of cells. The period during which such positive findings may be anticipated appears to be fairly limited; the survival times fixed in the present investigation—*i.e.*, 24, 48 and 96 hours and 8 days—therefore appear to be suitable. They were actually chosen on the basis of the aforegoing considerations.

In earlier studies on ECT in animals, the changes were also found—in those cases in which they could be observed—to be distributed throughout the brain but to be somewhat more marked in the frontal parts of the cortex.

Against the background of the aforegoing considerations, my search for irreversible nerve cell changes was focused on two phenomena, *i.e.*, shadow cells and neuronophagia, and the study was confined to the frontal parts of the brain.

A two-grade scale was used to evaluate the shadow cells. The first grade (slight) denotes that the cell damage was possibly irreversible; the second grade (more severe) denotes that the damage was probably irreversible.

The following schema shows how seldom such changes were found at the unbiased examination of specimens from the gyrus sigmoideus anterior, gyrus sigmoideus posterior and gyrus lateralis (*i.e.*, from the frontal parts).

Group	No. of shadow cells: possibly irreversible damage	No. of shadow cells: probably irreversible damage
C	5	—
A	6	2
B	7	3

If the extremely large number of nerve cells examined—several hundred in each specimen—is taken into account, the very small figures are remarkable.

True neuronophagia seemed to be still more rare. It was not observed in any of the control animals and could only be suspected on seven occasions in the animals subjected to ECT. This phenomenon should not be confused with so-called satellitosis. (Reference is also made to the photomicrographs.)

A number of earlier workers have reported disappearance of cells and acellular areas in the cerebral cortex of the experimental animals. I was therefore particularly interested in ascertaining whether such phenomena were present. I found that the cytoarchitecture consistently exhibited some irregularity. The nerve cells were assembled in groups, these groups appearing to have relationship to the vascular architecture. This irregularity seemed to be greater in the deeper layers of the cortex. It is therefore, in all probability, likely to be extremely difficult to determine the possible occurrence of small acellular areas or of an elective dropping out of cells. This has also been pointed out by Scholz[91], among others.

At the unbiased examination, I was unable to find any large necrotic areas. Single, small areas with *suspected* dropping out of cells were observed sporadically in animals subjected to larger series (11—16) of ECT's and with a longer survival time (group B). In only one specimen could this finding be considered as definite. In several cases, however, the suspicion could not be regarded as altogether unfounded. Finally, the following facts may be mentioned. It was a question of a few cells in 7 specimens out of the total 282 examined; the phenomenon was not found in any of the control animals. It was seen especially in group B.

The question of whether or not irreversible damage to the nerve cells may occur in association with ECT must therefore be answered in the affirmative. This is the first conclusion to be drawn from the observations reported. The changes found were not, however, extensive; they affected only a small minority of the nerve cells and occurred principally in those animals given the largest series of ECT's. On the other hand, only a very small proportion of the cells in the cerebral cortex were examined in the individual animal. In absolute figures, the number of damaged nerve cells in the whole cortex should be considerably greater. There is, however, no reason to anticipate a larger proportion of damaged cells in other sections of the cortex. On the contrary, it is possible that they would be less than in the frontal parts.

With regard to the animals given less intensive treatment—*i.e.*, 4 ECT's only—it may be concluded that it was not possible to demonstrate any irreversible nerve cell damage of any consequence.

The following statement may also be made. It appears almost impossible, in the case of animals surviving for a longer period after a series of ECT's, to recognize a dropping out of cells of this type and on the aforementioned scale. In my opinion, such cell changes can only be recognized if the microscopical examination is made in the course of the pathological process, *i.e.*, during the days immediately following the ECT. This emphasizes the necessity of choosing a suitable survival time for the animals in such neuropathological experiments.

CHAPTER 17

Considerations on the Pathogenesis of the Cerebral Changes

In the present experiments, the most easily discernible changes in the brain of the animals subjected to ECT were found in the vascular system. In every case the blood vessels were dilated and filled with blood. Since this also applied to the control animals, this phenomenon was not considered to be correlated

to ECT but was interpreted as an effect of the anesthesia in connexion with sacrificing the animals. Moreover, the hæmorrhages observed were mainly of the terminal type, presumably occurring during anesthesia and/or the operation immediately preceding death. They were, however, more common among the animals subjected to ECT ($p < 0.01$) thus indicating that ECT resulted in an increased susceptibility to terminal hæmorrhage.

A more specific process, denoted as changes in the vessel wall, was also noted; it included phenomena associated with the process of repair. This reaction showed a more distinct relationship to ECT—the difference between the control animals and those subjected to ECT being significant, $p < 0.01$—and appeared less likely to be attributable to age. It also exhibited a correlation to the survival time, a fact which provided an additional reason for ascribing the reaction directly to the electric shock treatment.

Thus, it was presumably not an acute œdema of the terminal type, but an œdema of somewhat earlier date. The estimated time of its onset is in good agreement, from the point of view of time, with the ECT. It may be denoted as a certain stage of cerebral œdema following an injury to the walls of the small blood vessels in the brain. The electric shock treatment appeared to be directly responsible for this injury. It is presumably largely reversible within a limited period of time—days only. While it is in progress, there appears to be an increased risk of more severe changes on exposure to further noxæ. This vascular injury provides a natural explanation of the apparent effect of ECT as a predisposing factor for the occurrence of terminal hæmorrhage. But, the actual injury only became discernible later, when it had reached a more reparative stage. Reference may also be made to the observations of Bjerner, Broman & Swensson[8] in experiments with infusions of trypan blue, namely abnormal passage of the stain through the blood-brain barrier after ECT (*cf.* Table 1).

A generalized glial reaction of progressive type appeared, from the point of view of time, to be parallel to the reparative element in the vascular changes. There was a difference ($p < 0.01$) between the control animals and those subjected to ECT with regard to the former phenomenon. When the material was divided into younger and older animals, respectively, this difference was still found in the latter category. This fact indicates that both ECT and a higher age may contribute to the occurrence of a glial reaction of this type. This is scarcely surprising in view of the strong tendency of the glia to react to various noxæ and of its importance as reparative tissue. But, it also indicates that the glial reaction is more unspecific; it therefore appears inadvisable to refer the changes observed *exclusively* to a particular injurious agent. However, in the present experiments, it appears plausible to ascribe the higher incidence of a glial reaction in the animals subjected to ECT mainly to the effect of the treatment, and as associated with a phase of repair.

Changes in the form of focal accumulations of nerve cells in different stages of chromophobia were observed in the brain of the animals that had undergone ECT. They were found more frequently in these animals than in the controls ($p < 0.01$). Age appeared to play a subordinate rôle.

Focal accumulations of altered cells have often been observed in various neuropathological conditions. Spielmeyer[97] expressed the opinion that, associated with a particular type of nerve cell changes, they could be denoted as highly specific to ischæmic injuries. They have also been found by several workers —although in a more marked form—in experimental studies with cerebral ischæmia on animals. Reference is only made here to the experiments of Gildea & Cobb[35] with cats and those of Grenell[38] with dogs. In both cases ischæmia was induced by ligating the blood supply to the cerebrum. It is evident from both investigations that the damaged nerve cells most frequently had a focal distribution. The changes were irreversible in some cases and the nerve cells showed a tendency to disintegration, with resulting acellular areas or areas of devastation. A survey of the literature is given in both the aforementioned publications.

A number of authors have stated that such foci show a distinct relationship to the blood vessels. It may, however, be difficult to determine whether this is the case, since—as a rule—the specimens are seen in one plane only. MEYER[69], on the other hand, pointed out that perivascular cellular changes can scarcely be typical of ischæmia, but that in this condition the peripheral areas could reasonably be expected to be those most affected. Grenell[38] put forward another interpretation, *i.e.*, that the focal distribution is due to the fact that the nerve cells are arranged in groups which are not only anatomical but also metabolic units. It may also be recalled that Winkler & Potter[113], in their atlas of the brain of the cat, repeatedly stressed that the cells—at any rate the large pyramidal cells—often lie in groups of four to eight, seen in one plane. Such groups appear to receive their blood supply through particular capillaries; the angioarchitecture thus corresponds to the cytoarchitecture (PFEIFER[81]).

Judging by the modern literature on neuropathology, it now appears to be doubtful whether the aforementioned focal arrangement of the nerve cell changes should be regarded, to any great extent, as specific to ischæmia in the strict meaning of the term. This is because they have also been observed in association with a number of other injurious agents such as severe intoxication by carbon monoxide, cyanide, carbon dioxide, ether or insulin, and in vitamin B deficiency and malnutrition. If, however, the meaning of ischæmia is extended to include all states of disturbance in the oxygen supply (or nutrition) it would be natural to classify all the aforementioned noxæ under the same heading. But this would necessitate relinquishing the right to diagnose cerebral ischæmia in the strict sense of the term on the basis of the occurrence of a focal distribution of the nerve cell changes.

Thus, it may be concluded that the focal distribution does not permit any definite statements with regard to the pathogenesis of the nerve cell changes in question, although it speaks in favour of an ischæmic origin rather than against it.

Nor is the *type* of the nerve cell changes particularly specific. A breaking-down of the Nissl substance has been observed in such forms of neuronal stress as excessive work, excessive or inadequate stimuli and ischæmia. The work of Hydén especially has shown that a restitution of the Nissl substance takes place with the aid of the nucleus. Einarson expressed the opinion that nerve cells exhibiting peripheral chromophobia with coincident nuclear hyperchromatism represent a prolonged stage of activity. In the present experiments a similar explanation appears to be that such cells were in a reparative stage following neuronal hyperactivity, or that they were still affected by hypoxæmia (*cf. infra*). Thus, the type of the nerve cell changes also fails to provide any reliable interpretation of the pathogenesis.

The aforegoing considerations on the neuropathological changes may be summed up as follows. The changes cannot, *per se*, be regarded as pathognomonic of an ischæmic injury, but such an injury would provide a satisfactory explanation of their occurrence.

A study of the pathophysiological course should provide more information regarding the pathogenesis. From this point of view, there are presumably two possible causes of the neuropathological changes associated with ECT. Either the actual electric current or the epileptic seizure may be the injurious factor. The distribution of the current in the brain in ECT was discussed in Chapter 2. It was found that the greater part of the current was received by the integument and only a small proportion—about 5 per cent—by the brain. The quantity of electricity used in the present experiments did not exceed $6^1/_4$ joules in the individual case. The quantity of electricity passing through the brain was therefore so small that it would only raise the temperature in it by at most 0.003° C. The brain of the cat weighs about 30 grams. Even if the distribution of this thermal effect was not quite even throughout the brain, it would never reach such a level at any site that it could be considered as a possible pathogenic factor in the neuropathological changes. In view of the small quantity of energy, it does not appear reasonable to ascribe the neuropathological changes associated with ECT directly to the effect of the electric current. Its importance is presumably confined to inducing the epileptic seizure.

With regard to the epileptic seizure, the following injurious factors may be envisaged:

1. Generalized respiratory and circulatory cerebral hypoxæmia.
2. Focal angiospastic cerebral ischæmia.
3. Neuronal hyperactivity.

Various combinations of these factors may also occur.

It is an established fact that considerable hypoxæmia occurs in convulsions induced by electricity or by other means. Thus, HOLMBERG & LAHNE[45] showed, by oximetric determinations on man, that the arterial oxygen saturation decreases. Immediately after the onset of the seizure, there is a rapid fall, the saturation reaches a lower level of approximately 50 per cent and regains the normal level about $2^1/_2$ minutes later.

Silfverskiöld, in collaboration with Schmiterlöw[90], GORDH[37], HOLMGREN[46] and ÅMARK[94], has published several reports of studies on the circulatory conditions in ECT. It was demonstrated that the cardiac output and the venous reflow decreased. On the whole, the circulation underwent the same changes as in an extremely marked Valsalva experiment, with sharp rises in the blood pressure, on both the arterial and the venous side. All these features may be assumed to cause hypoxæmia. Moreover, apnœa is present during the seizure.

KREIENBERG[56] made parallel determinations of the blood flow through the carotid artery and the jugular vein in dogs. He found that every generalized epileptic seizure induced by ECT was accompanied by a sudden cessation of the blood flow through the cerebral vessels, succeeded by an increased flow.

It may therefore be assumed, on the basis of the aforegoing data, that generalized, transitory cerebral hypoxæmia occurs in ECT. It appears to be of relatively short duration.

Reference may be made to JUNG's[51] experiments to illustrate the order of magnitude of neuronal activity during the convulsions. He calculated the energy consumption during grand mal seizures in cats by means of amplitude measurements on the EEG. He stated that, during the seizure, it was on the average 10—50 times in excess of the normal consumption, the increase being greater in the cortex than in any other part of the brain (20—50 times the normal activity). Thus, during the seizure, as much energy would be consumed in somewhat more than 90 seconds as in approximately 30 minutes under normal conditions. Some conception of the increased output of cerebral activity is also afforded by the motor phenomena occurring in the course of the convulsion.

Summing up the observations made in various physiological experiments, it may be stated that a considerable increase in neuronal activity, with concurrent relative hypoxæmia, takes place during the seizure.

It nevertheless appears unlikely, on several grounds, that neuronal hyperactivity—either exclusively or mainly—could explain the neuropathological observations made in the present study. It is scarcely conceivable that only a minority of the nerve cells would take part in this activity, yet few of them exhibited changes. On the contrary, TOMAN *et al.*[105] pointed out that all the neurons could be assumed to partake in the output of energy during the convulsions. Therefore, if this factor is to be assumed to contribute to the

pathogenesis, it must reasonably only be in combination with some other factor and would then play only a minor rôle.

I shall return to this question in the discussion of the rôle of the time factor (*cf. infra*).

Light has been thrown on the question of angiospastic cerebral ischæmia during the convulsion by the investigations of Scholz & Jötten[92] (*cf.* p. 23). They demonstrated in cats that, after a series of ECT's in close succession, angiospastic ischæmia—focally accentuated—persisted in the cerebral parenchyma for 20—40 minutes. They ascribed this phenomenon to the effect of the convulsion and not to the electric current. Such angiospastic phenomena were earlier stated by Spielmeyer[98, 99, 100] to cause nerve cell changes following epileptic seizures. He also assumed that acute ischæmia precipitated the seizure. Later observations have shown that this can scarcely apply in ECT, owing to the short latency time, but that the angiospasm is instead a concurrent phenomenon.

The nerve cell changes found in my experiments appear, on the basis of the facts brought forward earlier, to be satisfactorily explained by an ischæmic origin. The occurrence of cerebral ischæmia in an epileptic seizure seems to be relatively well substantiated*. It is therefore reasonable to presume that the nerve cell changes were caused by ischæmia. The focal distribution of the changes might possibly argue in favour of a focal angiospastic mechanism (*cf. infra*).

As far as I can ascertain, the vascular changes observed may also be explained on the basis of an ischæmic disturbance.

In addition to the local association, an analysis of the time element should throw some light on the problem. If generalized hypoxæmia and neuronal hyperactivity during the seizure were mainly responsible for the nerve cell changes, these should not only be more widespread, but they should also be suspected to occur in more rapid succession to the seizure than was found to be the case. For, they reached a maximum only after 48 to 96 hours. With the staining method used, no generalized chromophobia (tigrolysis) could be observed in the nerve cells of the animals with a survival time of 24 hours only. A comparison may be made with Lorentzen's[65] investigation on the nerve cell changes accompanying insulin coma treatment. He found more or less generalized chromophobia even after a very short survival time. Some degree of tigrolysis may also be envisaged in ECT. However, judging by the results of Lorentzen, it would then be considerably less than in insulin coma.

Assessed on the basis of the present experiments, the nerve cell changes in ECT appear to differ somewhat in type from those in insulin coma, in that

* Reference is also made to Scholz's statements with regard to nerve cell changes observed in autopsy material from patients suffering from epilepsy.

they are more frequent and more delayed. This may plausibly be explained by the hypothesis that they are caused by the vascular disturbances (angiospasm and/or vasoparalysis) brought on by the seizures. Thus, they do not appear to be an immediate sequela of the convulsions, but a secondary one. The following must also be borne in mind. The changes observed were evidently a cumulative effect of several shocks; they were not discernible after a few shocks or a single shock. The fact that they only became visible after several ECT's does not, however, contradict the argument in favour of their vascular origin.

APPENDIX

FORM FOR THE PROTOCOLS

Part of the brain , Specimen no. , Cat no. , No. of ECT's , Survival time ,

Macroscopic. findings:

Microscopic. findings: (Encephalitic changes:)

Width of the vessels:

Changes in the vessel wall:

(Perivascular pigment:)

Hæmorrhages: No. Freshness: Free pigment: Glial reaction:

Width of perivascular spaces:

Width of pericellular spaces:

Acute terminal œdema:

Nerve cells:

Shrinkage:

Corkscrew appearance:

Spike-like processes:

Vacuolization:

Swelling:

Shadow cells:

Nearness to the vessels:

Areas of devastation:

Areas of glial reaction:

Chromaffinity:

General:

Focal:

Variability:

Nuclear hyperchromatism:

Homogeneization:

Glial reaction:

Neuronophagia:

Satellitosis:

Irreversible changes:

Notes:

Diagnosis: Date:

SUMMARY

1. The object of the present investigation is to determine, by means of experiments on cats, whether or not demonstrable cerebral changes result from epileptic seizures induced with electric current, *i.e.*, so-called electric shocks.

2. It is pointed out in the survey of the literature (Part One) that the results of earlier neuropathological animal studies on the effect of electric convulsive treatment (ECT) have varied appreciably. They have ranged from entirely negative findings to observations of highly important changes. It does not appear possible to explain these discrepancies entirely on the basis of such differences in the experimental technique as the species of animals and the size of the shock dose.

3. An account of the material and methods used in the present study is given in Part Two. The material comprises 31 cats subjected to ECT: 23 of them (group A) were given 4 ECT's and 8 (group B) 11—16 ECT's. The controls (group C) consist of 16 untreated cats. It is stressed that no difference appears to exist between the control group and the treated groups with respect to the age distribution.

4. The electric shocks were given with a condensor discharge (McPhail-Strauss plexacon apparatus). The shock dose was the lowest that sufficed to produce complete seizures and in no case exceeded $6^1/_4$ joules. The epileptic seizures were characteristic, in that they exhibited a latency time, tonic phase, clonic phase and coma stage. Epileptic seizures in cats thus strongly resemble those in man, with the exception that the total duration is, as a rule, shorter in the former case. An account is given of the phenomena occurring during the seizures and frequency diagrams for the duration of the respective stages are presented.

5. A uniform procedure was used for preparation of the material from the treated animals and the controls. The brain was removed from the living animal under anesthesia and specimens for histological examination—fixation in Carnoy's solution and staining with the gallocyanin-chromalum method—taken from the same parts of the brain in the whole material.

6. The specimens obtained were subjected to three separate examinations (I, II and III); part of the material also underwent a control examination (IV) —see further under point 9. The *1st examination* was intended to provide an orientation of the neuropathological phenomena in the animals subjected to

ECT and in the controls. A form for the protocols was drawn up on the basis of the findings (see Appendix). At the *2nd examination*, these observations were noted for each specimen and recorded in the protocols. It was found that certain changes in the walls of the smaller vessels, the glia and the nerve cells were present in the specimens from animals subjected to ECT (*cf.* point 7). These phenomena, although less marked, were also found in some of the controls. It was therefore necessary, in order to permit a comparison between the two categories of animals, to grade the phenomena in question. (This grading was actually made at the 2nd examination, but the results are not reported. This is because the origin of the specimens—*i.e.*, whether they derived from a treated or an untreated animal—was known; this must be assumed to imply a biased estimation.)

A *3rd examination* was made of newly prepared sections. Their origin (other than from what part of the brain they were taken) was unknown. This procedure is denoted as the unbiased examination. Specimens from the different groups of treated animals and from the controls were mixed together and examined concurrently. The observations made were graded and recorded on the forms. Finally, each specimen was diagnosed, *i.e.*, it was denoted as deriving from an animal subjected to ECT or from a control. Only after the diagnosis had been made were the covers removed from the labels and the numbers of the specimens recorded. A statistical analysis of the data recorded shows that there is a difference between the treated and the untreated animals. Thus, a collocation of the diagnoses obtained on examination of the six separate specimens from the isocortex results in a statistically significant difference ($p < 0.01$) between the controls and the treated animals. More intense treatment (11—16 ECT's) is found to result in more distinct changes than less intense (4 ECT's). The age also plays a rôle; the difference between the controls and the treated animals is most apparent in the older animals. The survival time is also of consequence; the changes are found to be most distinct when the survival time after the last shock is of medium duration (48—96 hours).

7. The various neuropathological phenomena that were found, at the unbiased (3rd) examination, to disclose the most marked differences between the treated and the untreated animals are, in the following order: changes in the vessel wall, nerve cell changes and a glial reaction of the progressive type. The two first-mentioned also show some relationship to age, although less marked than in the case of the glial reaction. The former must therefore be attributed mainly to the preceding ECT. This conclusion is also supported by the fact that the phenomena in question are most marked in the animals with a medium survival time (48—96 hours) after ECT and in those given more intense treatment (11—16 ECT's). The differences between these groups and the control group are significant ($p < 0.01$).

The *vessel wall changes* found more frequently and more distinctly in the animals subjected to ECT consist of characteristic, sac-like dilatations of the perivascular spaces, which in some cases contain histiocytic elements. The *glial reaction*, of the progressive type, consists of an increase in the number of the smaller glial elements in the parenchyma and of satellitosis beside the nerve cells. The *nerve cell changes* observed are in the form of various stages of chromophobia, frequently with coincident nuclear hyperchromatism. The arrangement of such cells is mainly focal.

A difference ($p < 0.01$) is also noted between the treated and untreated animals with respect to the occurrence of pigment of the autogenic type in the perivascular spaces.

8. The problem of sources of error and the importance of possible artefacts for the results of the examination are discussed in Part Four. It is stressed that the differences noted between the control animals and those subjected to ECT cannot be explained on the basis of post-mortem changes, fixation artefacts, variations in the intensity of staining, of thickness of the sections or of their area. The sources of error dependent on variations in these experimental conditions may be reduced by two means. One is a standardization of the method and the other is the treatment of specimens from the controls and the treated animals concurrently. With respect to the area of the sections, a special analysis shows that no difference of any statistical significance exists between the specimens assigned a positive and a negative diagnosis, respectively.

The choice of the animals and of the specimens from the brain of the individual animals is also discussed. Particular attention is focused on the age distribution of the animals in the various groups, and on the possible occurrence of spontaneous pathological phenomena of the encephalitic type. The parts of the brain selected for the examinations—the same for all the animals—may be considered as representative of the isocortex, in particular of its frontal part.

9. It has been found in earlier studies that, in neuropathological investigations of this kind, the personal estimation of the specimens is subject to special sources of error of a subjective nature. In order to avoid such sources of error, a control series was set up and the examinations made without previous knowledge of the origin of the specimens. The results were submitted to statistical analyses. The combined use of these procedures seems to be a new approach to the problem.

As a further check, a *control examination* (4th examination) was made. This was confined to 8 control animals and 8 subjected to ECT. The original numbers on the specimens were altered by an impartial pathologist and the list of new numbers retained by him. New sections were cut and stained (with the same histological technique as earlier). The task was then to determine,

by means of examination of three pairs of specimens from the brain of each of the animals, which of these animals had undergone ECT. This resulted in 7 out of the 8 control animals being diagnosed as controls (although one of them with some doubts). Of the 8 treated animals, 7 were diagnosed as treated (3 of them with some doubts). On the basis of the findings, 1 of the 2 remaining animals was denoted as presumably shocked and the other as presumably a control: this proved to be correct. Thus, no definitely incorrect diagnosis was made in the case of any of the animals. On the hypothesis that this distribution was purely random, the probability of classifying the animals correctly is less than 0.0001. The diagnosis was doubtful in two cases: if they are not included, the corresponding probability is approximately 0.0003. The result thus substantiates the reliability of the method.

10. In the case of the individual neuropathological phenomena—changes in the vessel wall, the glial reaction (including satellitosis) and changes in the nerve cells—considered separately, the results of the earlier unbiased examination (III) could be reproduced at the control examination (IV). The agreement is shown in statistically significant differences between the animals subjected to ECT and the controls. It is unaffected by the fact that the scale of values had changed with time—the two examinations were made with an interval of several months. The differences between the two categories of animals are still apparent.

11. On the basis of the present results, the question of whether or not irreversible nerve cell changes may occur after ECT must be answered in the affirmative. Such changes that may be considered with any degree of certainty to be irreversible—*i.e.*, shadow cells and neuronophagia—are only present in a small minority of the nerve cells, and mainly in those animals given intense treatment (11—16 ECT's). The incomparably greater proportion of changes in the vessel wall, the glia and the nerve cells may therefore be regarded as reversible.

The pathogenesis of the changes observed is discussed. Cerebral ischæmia in connexion with the epileptic seizure is suggested as the most probable causative mechanism.

12. More intensive and objective methods, *i.e.*, ultra-violet and X-ray microspectrography, are now available for neurocytological studies (*cf.* Preface). Before such methods can be applied to cerebral changes of a more diffuse nature, a more extensive method is necessary to determine when they can be applied. It is suggested that the method used in the present investigation may be of use as a scouting method in such cases.

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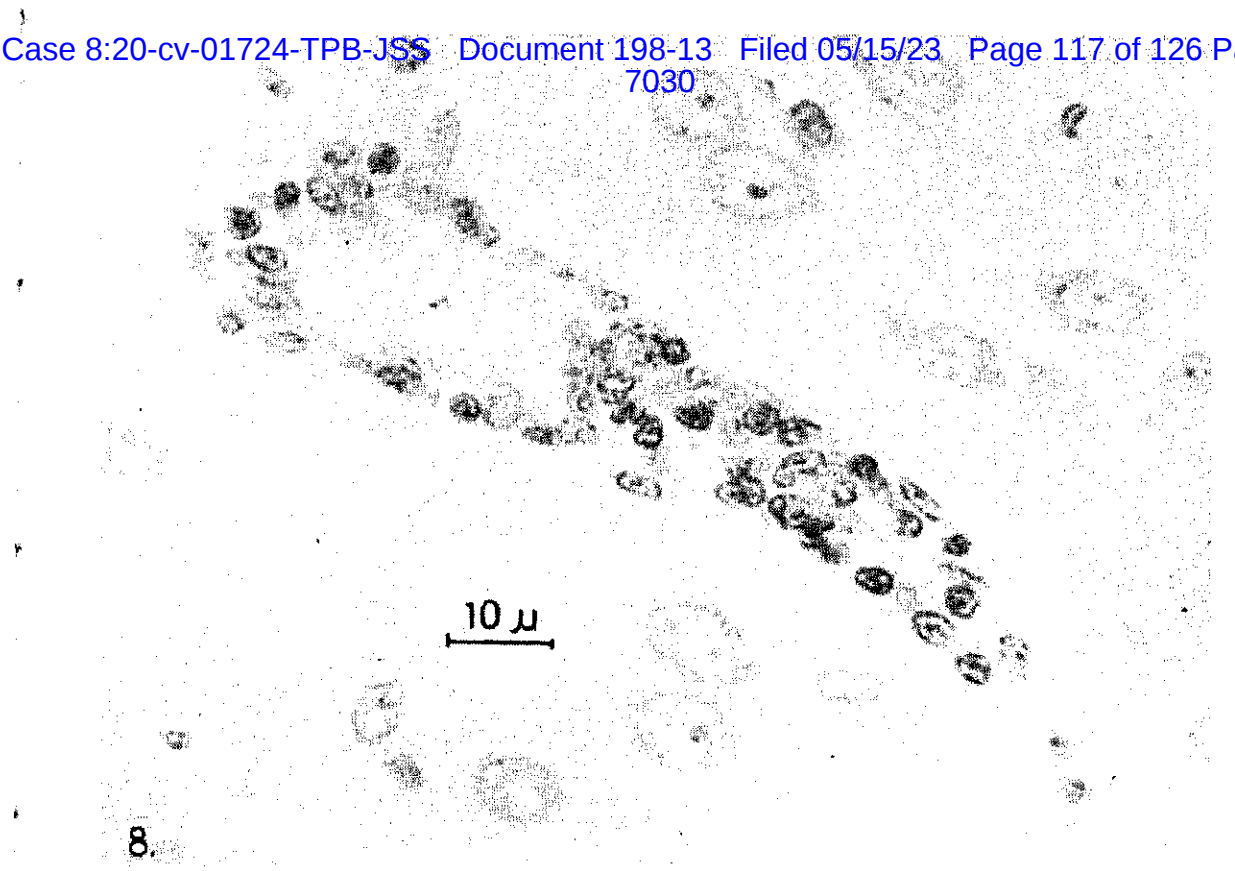

Figure 8. Specimen no. 735. Gyrus sigmoideus anterior sinister, cat no. 60, 4 ECT's, survival time 24 hours. Small round-cell infiltration beside a blood vessel (encephalitic).

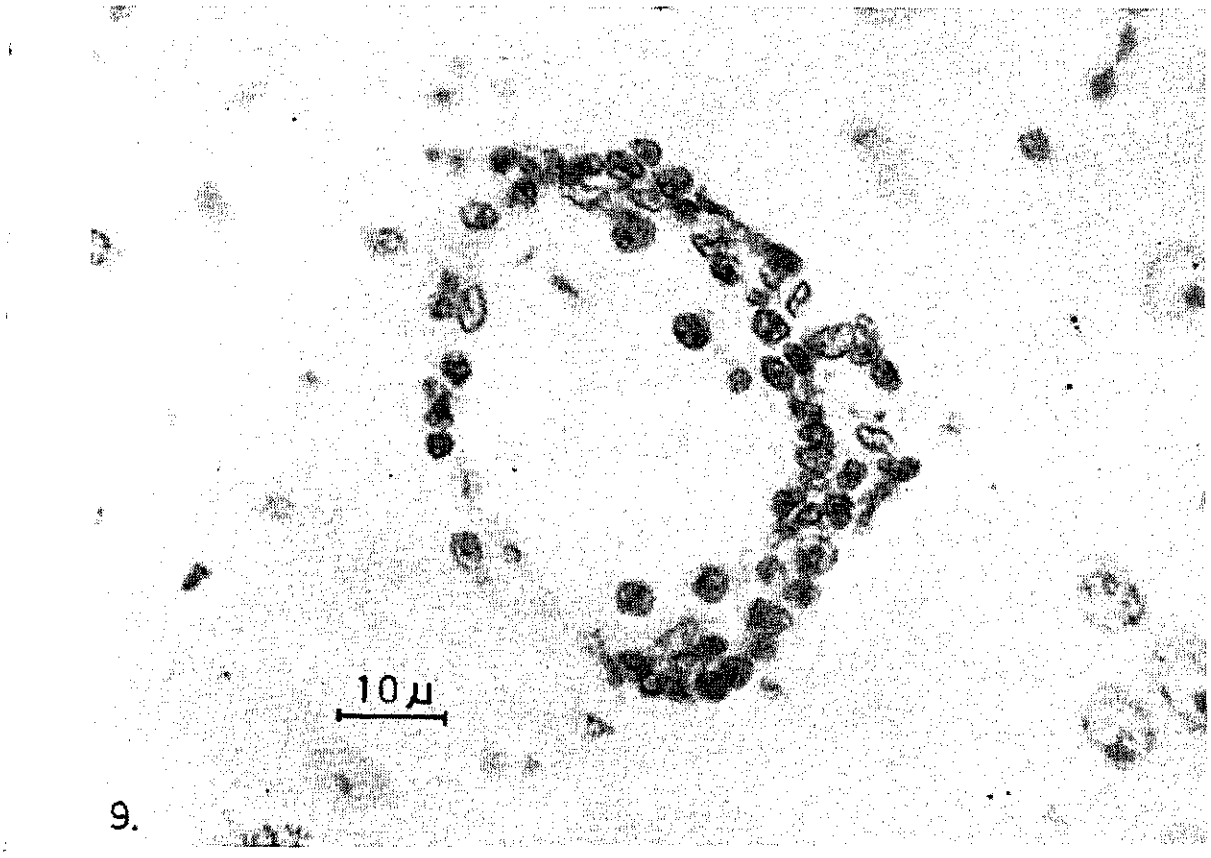

Figure 9. Same as figure 8.

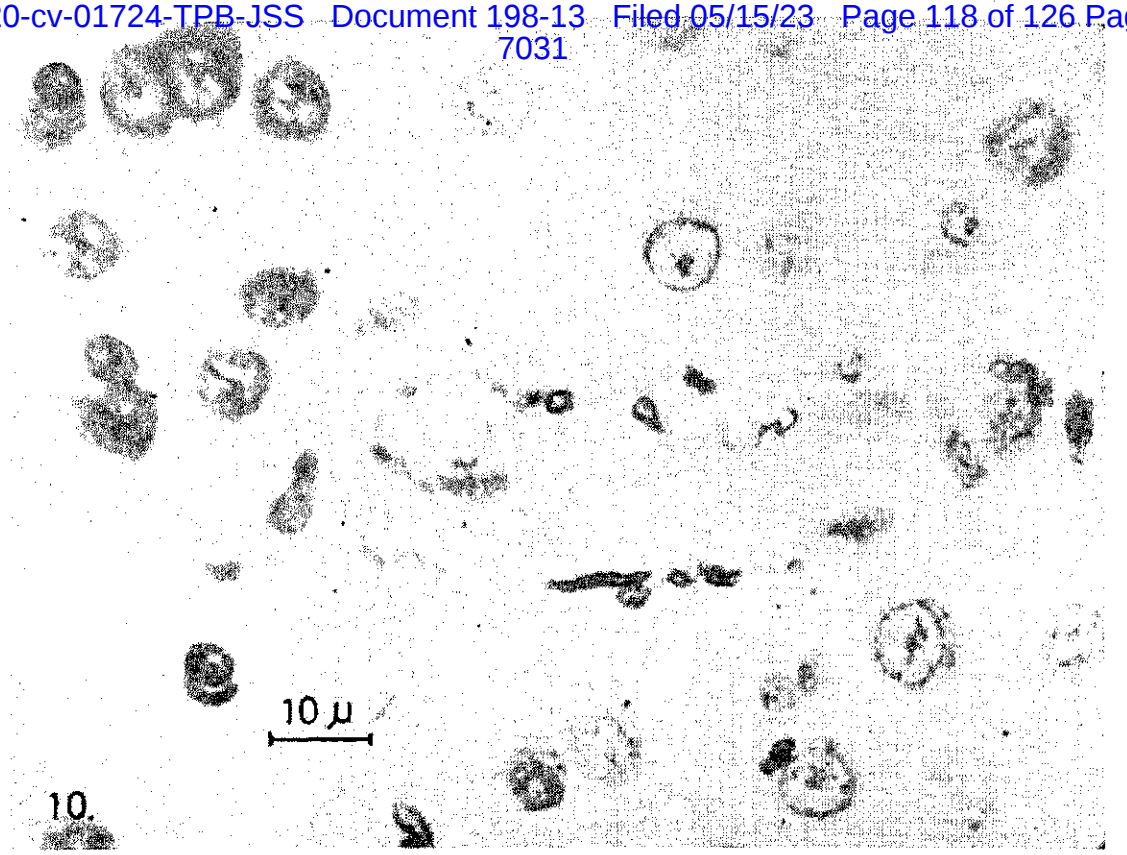

Figure 10. Specimen no. 298. Gyrus sigmoideus posterior dexter, cat no. 27, 11 ECT's, survival time 96 hours. Dilated perivascular spaces of irregular shape, forming sacs.

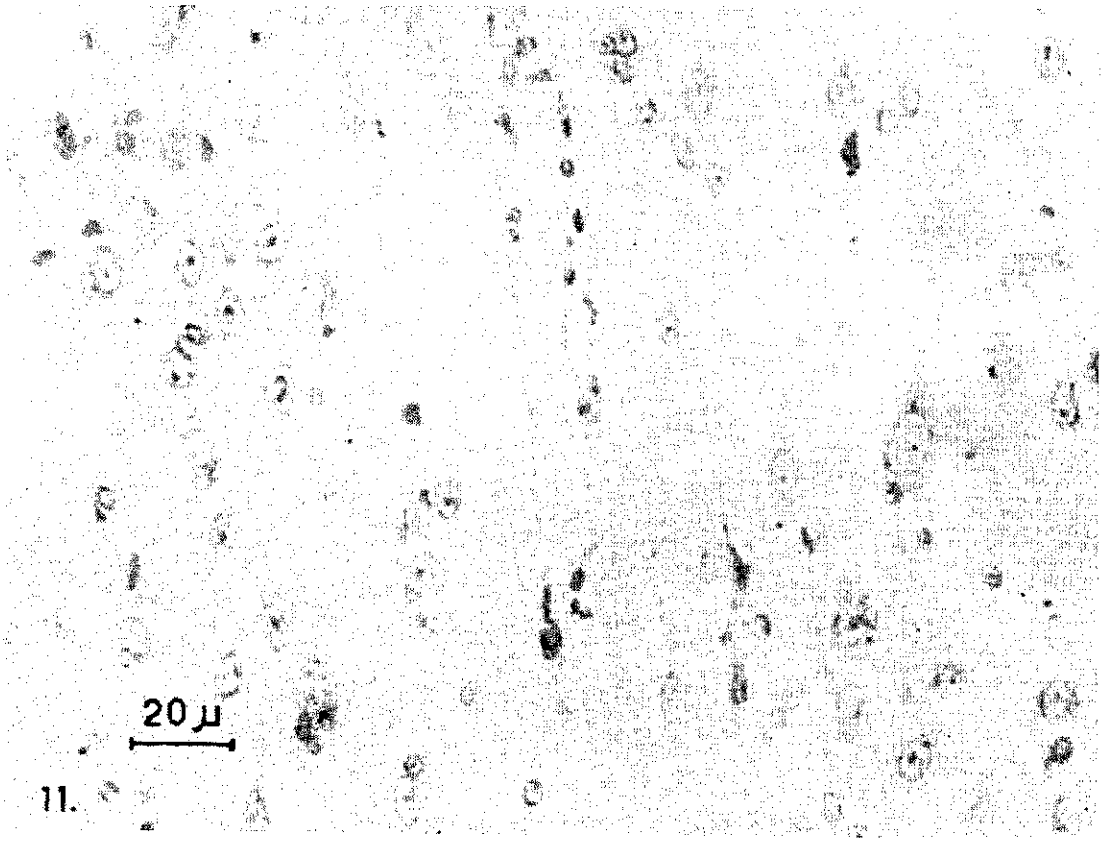

Figure 11. Specimen no. 293. Gyrus lateralis sinister, cat no. 27, 11 ECT's, survival time 96 hours. Dilated perivascular spaces of irregular shape.

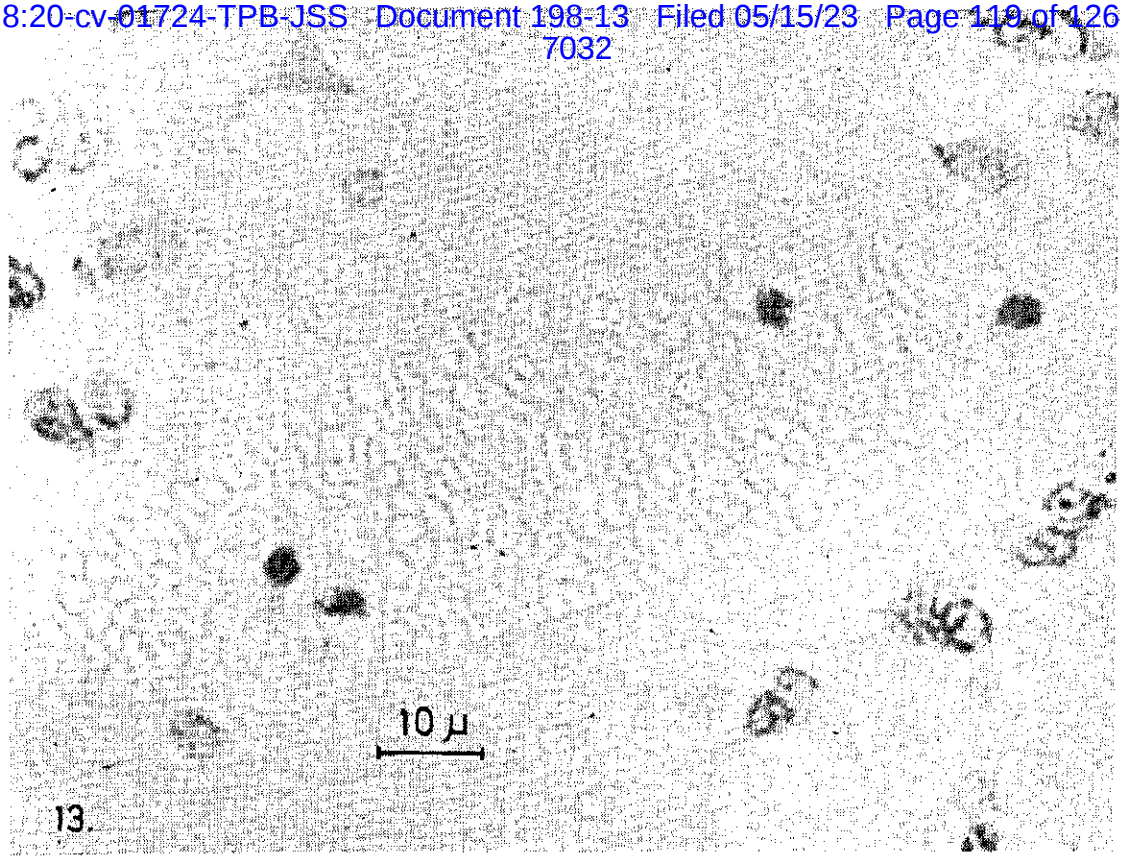

Figure 13. Specimens no. 299. Gyrus lateralis dexter, cat no. 27, 11 ECT's, survival time 96 hours. Terminal hæmorrhage, unchanged erythrocytes, no glial reaction.

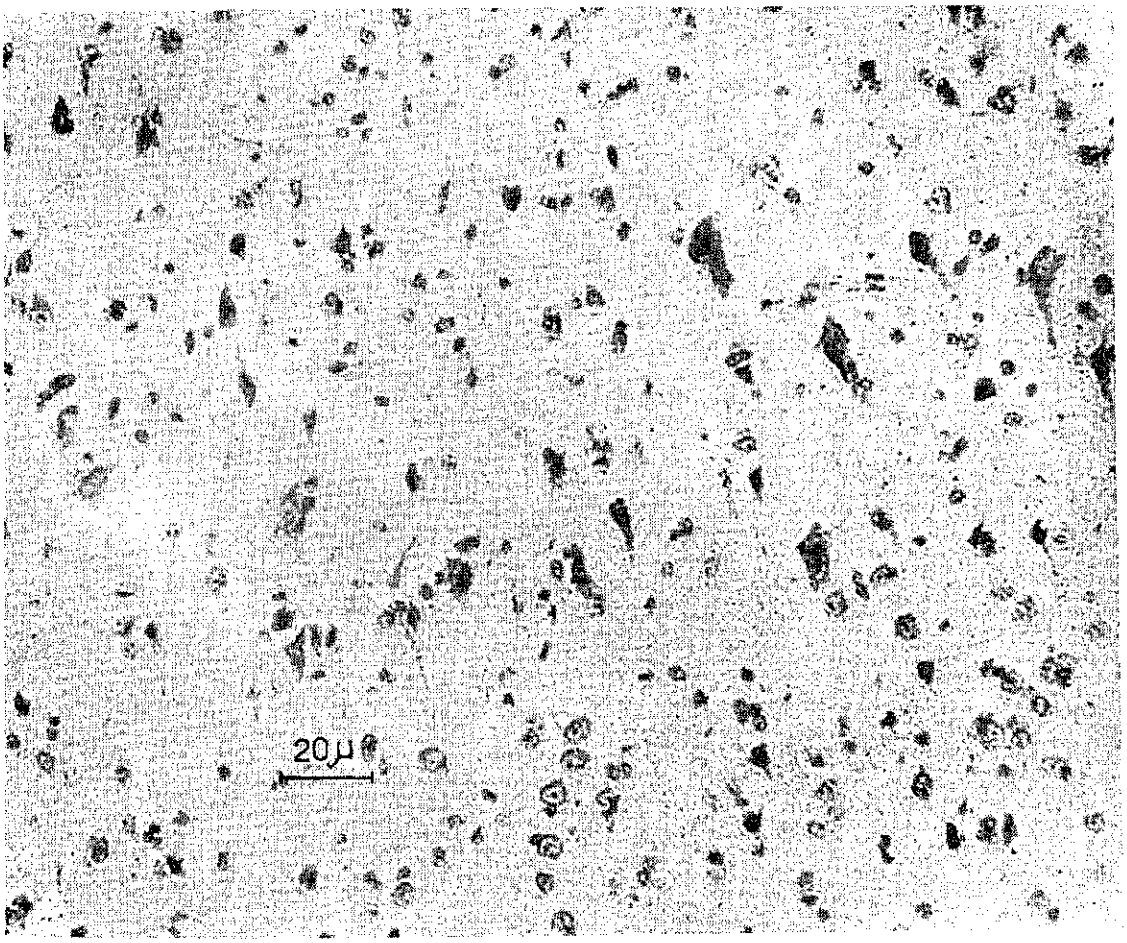

Figure 14. Specimen no. 683. Gyrus sigmoideus posterior sinister, cat. no. 56, 4 ECT's, survival time 24 hours. Variability of nerve cells, focal arrangement, fragmentation.

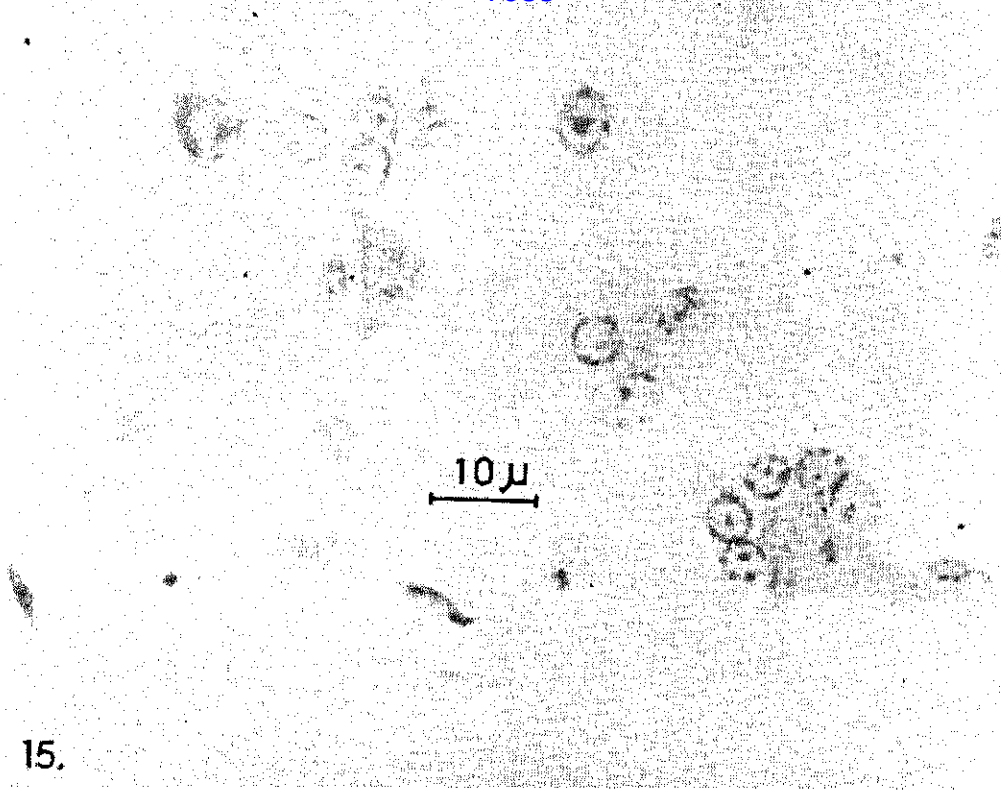

Figure 15. Specimen no. 757. Gyrus sigmoideus posterior dexter, cat. no 61, 4 ECT's, survival time 8 days. Satellitosis.

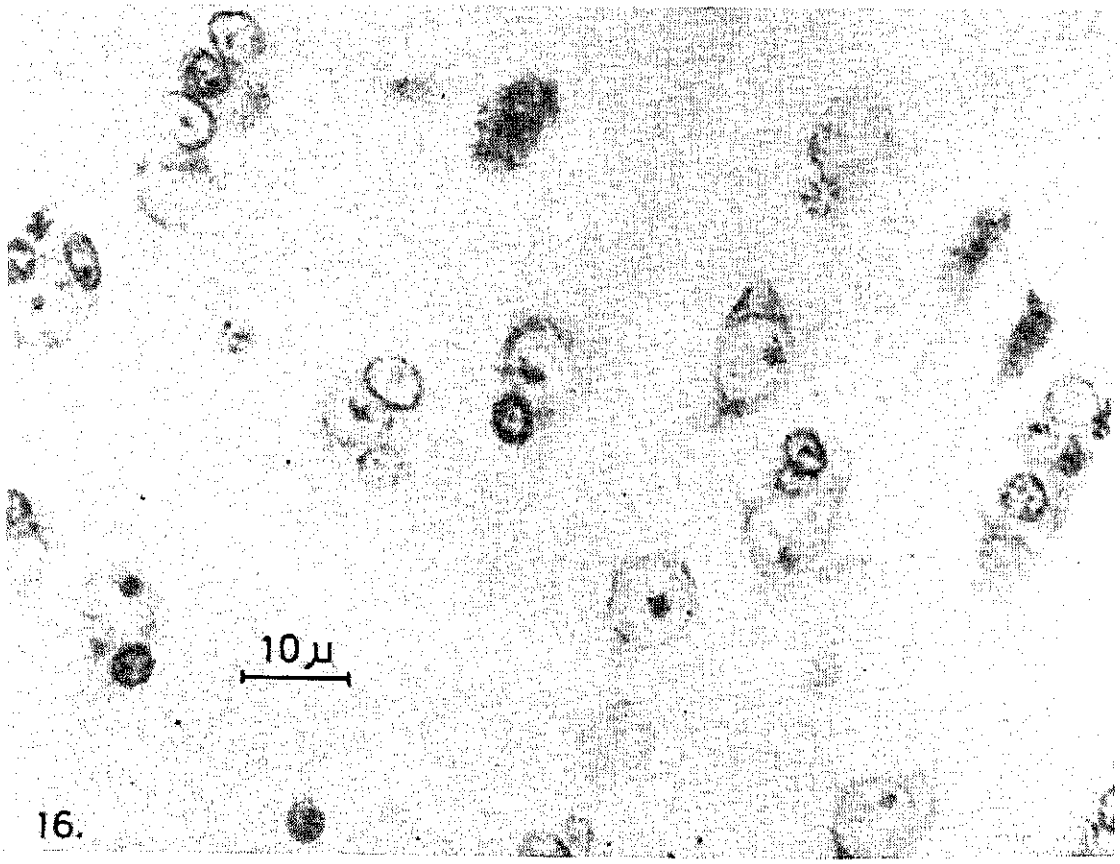

Figure 16. Specimen no. 469. Gyrus sigmoideus posterior sinister, cat no. 41, 16 ECT's, survival time 8 days. Variability of the nerve cells in the deeper layers (V).

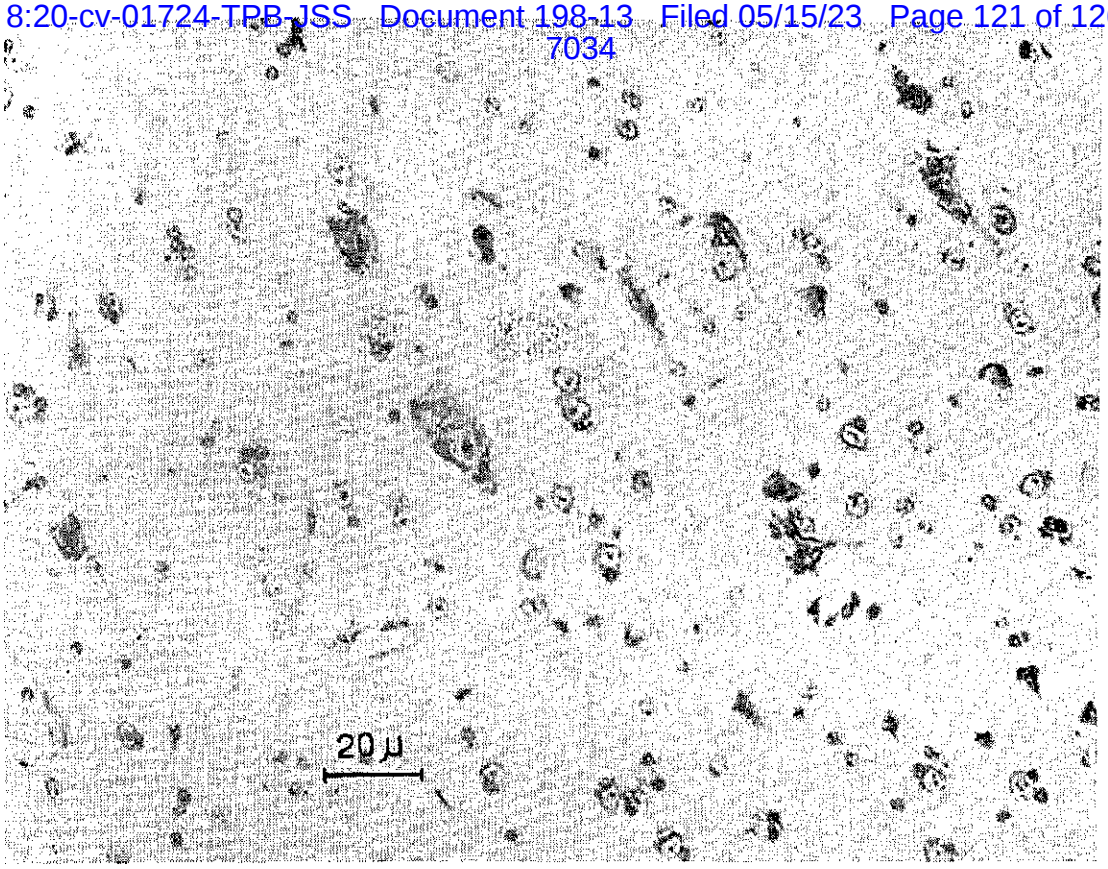

Figure 17. Specimen no. 825. Gyrus sigmoideus anterior dexter. Cat no. 66, 4 ECT's, survival time 48 hours. Variability of nerve cells, chromophobia, a shadow cell.

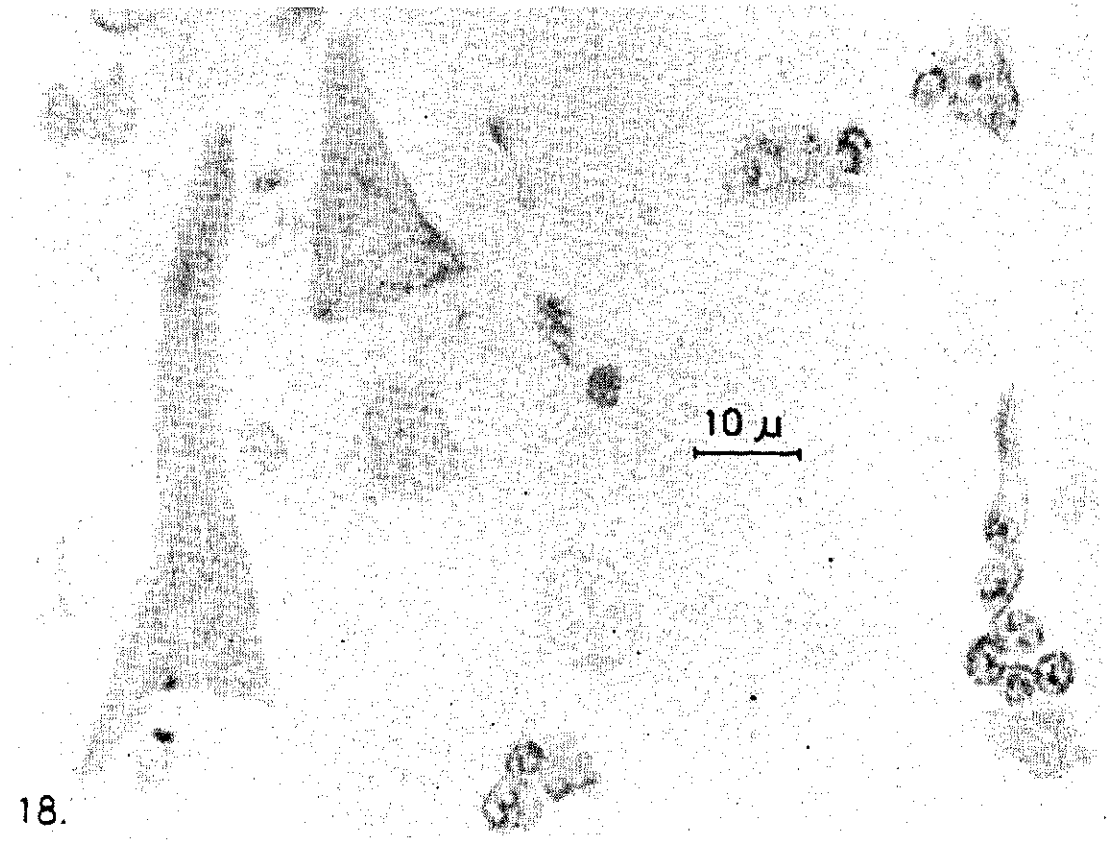

Figure 18. Specimen no. 356. Gyrus sigmoideus dexter, cat no. 30, 16 ECT's, survival time 96 hours. Satellitosis.

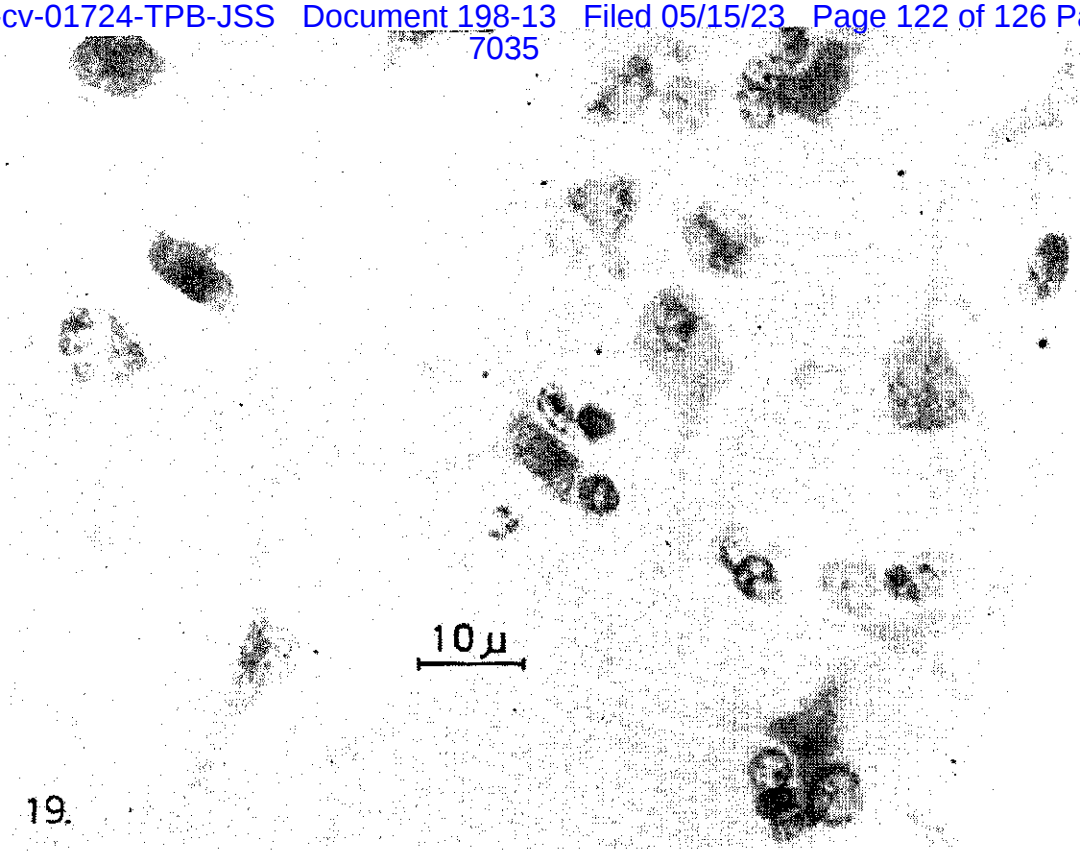

Figure 19. Specimen no. 298. Gyrus sigmoideus posterior dexter, cat no. 27, 11 ECT's, survival time 96 hours. Variability of the nerve cells, chromophobia in the cytoplasm, nuclear hyperchromatism. Satellitosis.

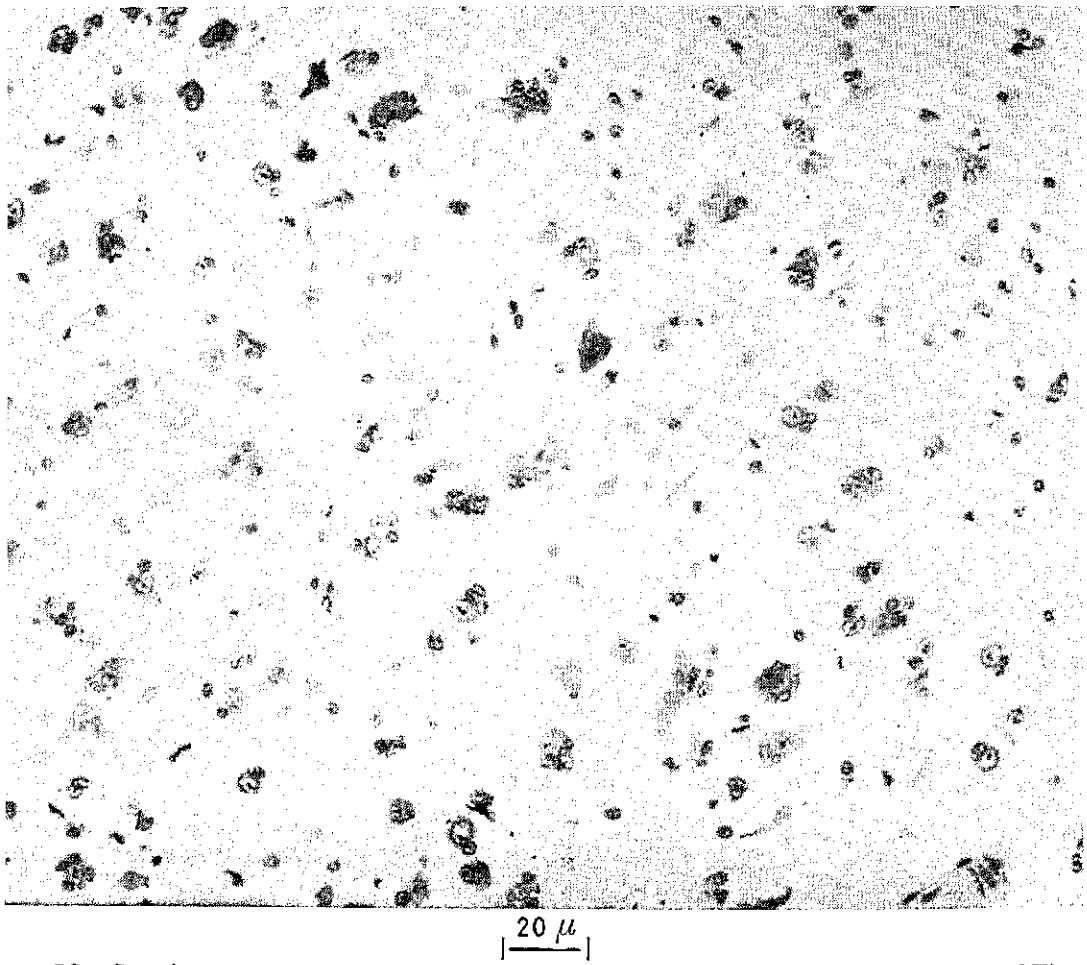

Figure 20. Specimen no. 832. Gyrus sigmoideus anterior sinister, cat no. 67, 4 ECT's, survival time 48 hours. Variability of nerve cells, focal arrangement.

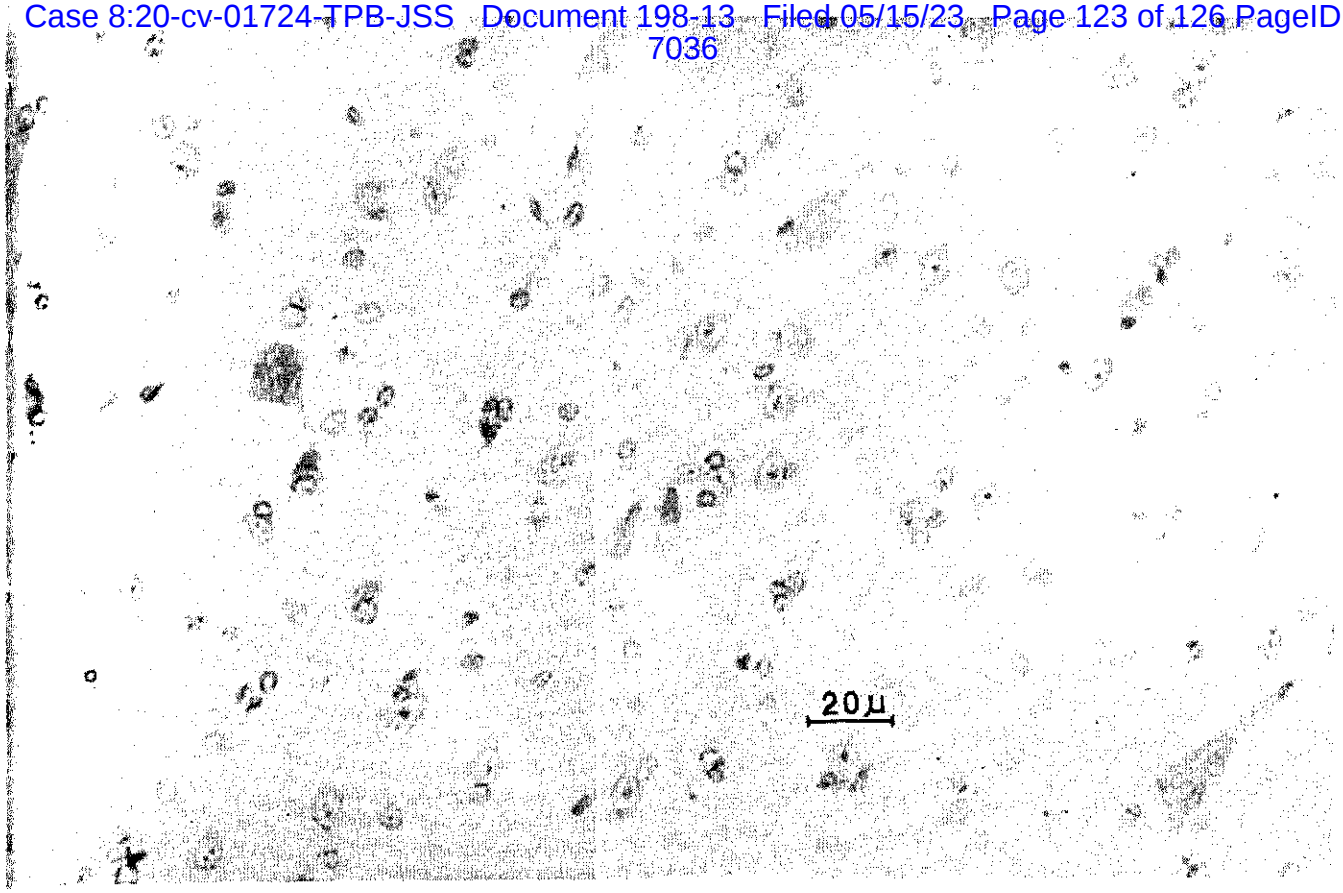

Figure 21. Specimen no. 717. Gyrus lateralis dexter, cat no. 58, 4 ECT's, survival time 24 hours. Variability of the nerve cell picture, slight glial reaction in the parenchyma.

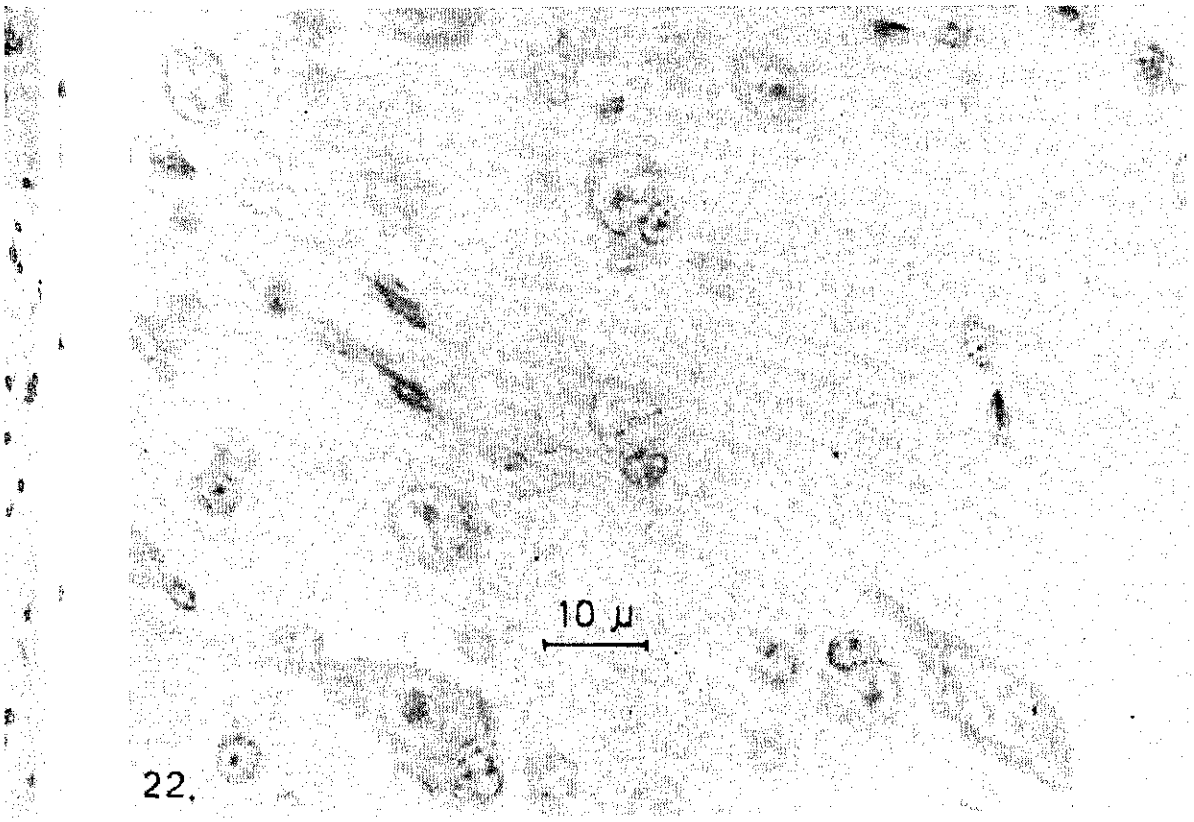

Figure 22. Specimen no. 541. Gyrus lateralis sinister, cat no. 46, control animal. Nerve cells of distinct structure in the vicinity of a small vessel.

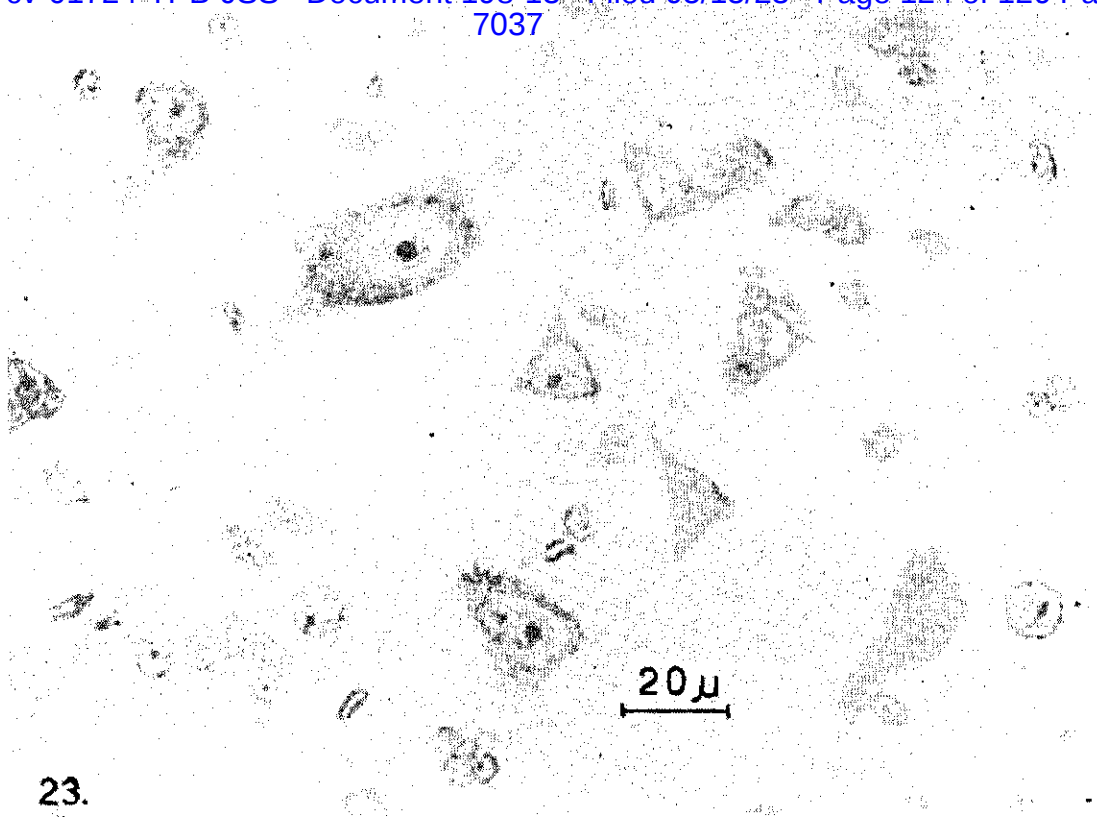

Figure 23. Specimen no. 526. Gyrus sigmoideus posterior sinister, cat no. 45, control animal. Nerve cells of distinct structure, normal.

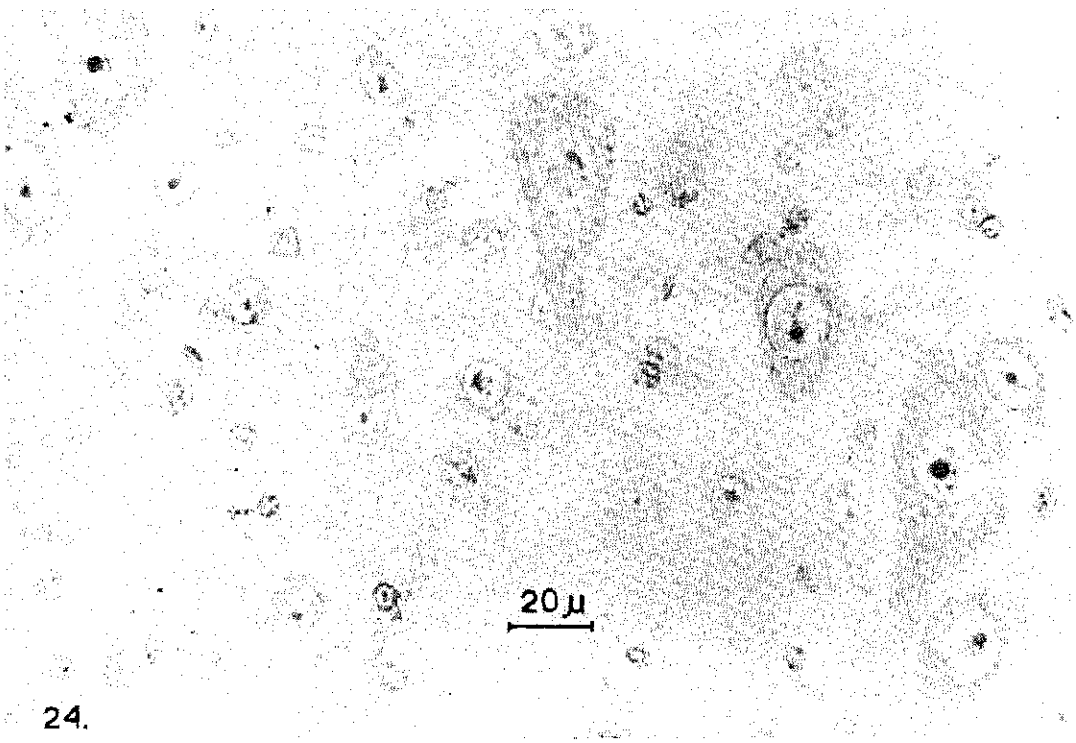

Figure 24. Same as figure 23.

Table I.

Author Year	No. and Species of Animals	Age of Animals	No. of Control Animals	Electrical Data: Voltage Volts	Electrical Data: Flowing time Sec.	Electrical Data: Current Milliamp.	Electrical Data: Electrodes	The Shocks: No. of Shocks	The Shocks: Type of Convuls.	The Shocks: Frequency	Method of Sacrificing	Survival Time	Fixation Medium
CERLETTI BINI 1940	Dogs 12	Not stated	5	≥ 125 a.c.	2—5	300—3000		20—40	Complete	1—2/day	Electrocution	5 days—5 months	96 % alcohol
	8	Not stated		≥ 125 a.c.				20—70	Complete	Intervals 3—5 min.	Electrocution		96 % alcohol
	14	Not stated		110—220	17—50	900—2500			Complete		Only two survived the shocks		96 % alcohol
HEILBRUNN TIEBERT 1941	Rabbits	Not stated	Not stated	80—90	Not stated	Not stated	Not stated	Not stated	Complete	Single	Aspiration biopsies	2—60 min.	Not stated
BARRERA 1942 LEWIS PACELLA KALINOWSKY	Monkeys 12 Macacus rhesus	Not stated	Not stated	75—135 a.c.	0.10—0.15	Not stated	Not stated	Up to 30	Complete & abortive	3/week	Air embolism	24 hours	Not stated
ALPERS HUGHES 1942	Cats 30	Not stated	0	110 a.c.	Not stated	150—200	Small 5 mm diam.	10—23	Complete	3—6/week	Section of the carotid artery	Not stated	Dilute formal-dehyde
HEILBRUNN WEIL 1942	Rabbits 28 Rats 10	Not stated	0	60—150	0.30—0.50	65—300	2 cm²	1—13	Typical	2—3/week		0—32 days	
BJERNER BROMAN SWENSSON 1944	Rabbits 4	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	1—10	Complete	Intervals 3 min.	Bleeding under ether anesthesia	Short	20 % formalin
NEUBUERGER WHITEHEAD RUTLEDGE EBAUGH 1942	Dogs 12	Not stated	0	80 a.c.	0.15	200	Not stated	2—25	Complete & abortive	Intervals 3—5 days	Bleeding under anesthesia. Some died during ECT	0—129 days	Not stated
GLOBUS HARREVELD WIERSMA 1943	Dogs 7	Not stated	1		30 sec. + 15—75 minutes	200—700		1—11	Electro-narcosis		Ether. Electrocution	Minutes—2 months	96 % alcohol, 10 % formalin
LIDBECK 1944	Dogs 3	Not stated	0	Not stated	0.2—1.0	250—500	Not stated	14—16	General	2—7/week	Shot through the heart	Short	10 % formalin, 70 % alcohol
ALEXANDER LÖWENBACH 1944	Cats 23	Not stated	Previous material. Not stated	120—550	0.2—10	60—2000	1 cm²	Single in 19 cats	Mostly complete		Decapitation	≤ 30 minutes in 16 cats. Up to 9 days	10 % formalin
WINKELMAN MOORE 1944	Cats 12	Not stated	0	Faradic — 70—80	0.1—1.0	300—400	Fronto--occipital 2.5 cm diam.	2—17	Complete	3/week	Bleeding under anesthesia	3 days—10 weeks	4 % formalin
FERRARO ROIZIN HELFAND 1946	Monkeys 10 Macacus rhesus	Not stated	0	70—90	0.1—0.4	102—400	Small & Large	4—18	Complete	3/week	Ether	12, 24 & 48 hours	95 % alcohol, 10 % formol, formolbromide
FERRARO ROIZIN 1948	Monkeys 11 Macacus rhesus	Not stated	Not stated	90	0.3—0.5		Small 2 cm² Large 14 cm²	32—100	Complete	Consecutive	Ether	½ hour—1½ years	95 % alcohol, 10 % formol, formolbromide
MASSERMAN JACQUES 1948	Cats 6	Not stated	2	30	5		4 cm²	10	Complete	Intervals 2—3 days	Removal of brain in live cat, anesthesia	1 day—12 weeks	Formalin
SIEKERT WILLIAMS WINDLE 1950	Monkeys 5 Macaca mulatta	Not stated	1	90	0.1—0.4	90—310	7 mm diam.	13—19	Complete	3/week	Perfusion under anesthesia	1—2 days	10 % formalin in colloidal acacia

Earlier Experimental Studies on ECT in Animals

Histological Technique		Macroscopical Observations	Neuropathological Observations in the Treated Animals			Ditto in the Controls	Authors' Interpretations and Comments
Fixation Method	Staining		Vascular Changes, Hæmorrhages	Glial Changes	Neurocytological Changes		
Immersion	Nissl, Marchi, Bielschowsky, Spielmeyer iron htx, Hortega carbonate, Alzheimer IV, Daddi-Herxheimer, Pal-Kulschitzky etc.	0	0 hæmorrhages. Some œdema. Tortuous capillarics. Pigment bodies perivasc.	Small changes of progressive type.	II—IV layers vacuolization of the cytoplasm. V—VI layers pyknotic retraction.	Pyknosis, less marked than in the treated animals.	Small changes not of destructive nature. Only slight œdema. No severe cellular changes. Pyknosis reversible, but no artefact.
Immersion		0	Œdema.	Regressive changes.	Apical hyperchromatism. Slight chromatolysis.		Presumably reversible changes.
Immersion		0	Erythrocytes, leucocytes & pigment in perivasc. spaces.	Mostly regressive.	Severe changes (deeper layers). Acellular areas.		Irreversible changes.
Immersion	Cresyl violet.			Slight swelling of astrocytes & oligodendrocytes.	Loss of sharp outline of ggl cells. Disappearance of Nissl bodies. Swelling of the cells. Vacuoles.	No changes	The authors stress that the procedure did not produce any artefacts in the control animal biopsies.
Immersion	Stainings for nerve cells, myelin sheaths, axis cylinder, glia; htx-eosin.	0	0	0	Shrunken with pyknosis of the nucleus, paling of cytoplasm, disappearance of the Nissl substance. Shadow cells. Focal distribution.	Same changes as in the treated animals.	The changes could therefore not be related to the seizures. No evidence to indicate a relation between ECT and histopathological changes, which could be detected with usual techn.
Immersion	Toluidine blue, cresyl violet, H & E, Ponceau B, Weil.	Congestion of the pial vessels	Subarachnoid hæmorrhages in 14 cats. Ditto in the brain in 9 cats.	Only in the vicinity of the hæmorrhages. One glial nodule.	Normal conditions.		"Hæmorrhages is the common lesion".
	Aniline dyes, iron htx, van Gieson, Azan, Weil.		Localized hæmorrhages in the pia-arachnoid. Perivenous & pericapillary hæmorrh. in the parenchyma.	No glial proliferation; only in the vicinity of the hæmorrhages.	No generalized ganglion cell disease. Shrinkage and hyperchromatism in the vicinity of the hæmorrhages.		16 rabbits paralyzed in the hind legs and the urinary bladder in the course of the ECT. All animals lost weight.
Perfusion	Infusion of trypan blue in living animals.		No hæmorrhages but pathological passage of the stain through the blood-brain barrier.		Not sought for.		Less changes after ECT than after insulin coma and cardiazol shocks.
Immersion	Nissl, htx-eosin, Loyez, Cajal, Hortega.		Congestion, vascular dilatation, minute hæmorrhages in the cortex.	Satellitosis and neuronophagia occasionally.	Rather widespread damage: tigrolysis, paleness, swelling, vacuolization, even ischæmic and severe changes. Ghost cells.		Many of the changes reversible. Definitely pathological changes but not to be regarded as serious.
Immersion	Nissl, Weil, Herxheimer scarlet red, Bielschowsky, Spielmeyer, Globus.	0	0	0	0	0	Electronarcosis needs strong current for long time. Thus the current itself no injurious factor. No changes even in the electrocuted animals.
Immersion	Nissl, htx-eosin, Mallory, Van Gieson, Spielmeyer, Holzer.	0	Single perivascular hæmorrhages; capillary thrombi.	0	Nerve cells shrunken throughout with decrease of stainable granules. A single area of increased shrinkage. Few ghost cells.		Pathological alterations minimal.
Immersion	Cresyl violet, Masson, Bodian, benzidine.		With strong current 500—1800 m.a. vasoconstriction in the path of the current. With 2000 m.a. vasoparalytic stasis.	0	Pathological changes only with current of $\geq$ 1800 m.a. & reversible. With $\geq$ 2000 for 5—10 sec. irreversible changes, shrinkage and severe pyknosis.	0	
Immersion	Htx-eosin, Weil, toluidine blue, scarlet red, Bodian, Cajal, Hortega.	0	With excessive doses a small pericapillary hæmorrhage and slight congestion in two animals.	No changes.	No changes.		Also serial sectioning. With doses analogous to those given in humans no morphologic changes.
Immersion	Nissl, htx-eosin, Hortega, Cajal, Roizin, Bielschowsky, Bodian, sudan III.	0	Increased permeability of the vessel walls, distended perivasc. spaces, some diapedesis. Pigment in the intima.	Satellitosis. Neuronophagia. Slight proliferation near the vessels.	Tigrolysis & chromatolysis, scattered areas of rarefaction but mainly reversible changes, swelling, pallor and some reduction in number of cells perivasc.	0	The changes are most pronounced in the path of the current, and more pronounced in parts surrounding the blood vessels.
Immersion	Nissl, htx-eosin, Hortega, Cajal, Roizin, Bielschowsky, Bodian, sudan III, sudan black.	Only slight congestion in few animals	Increased permeability of the vessel walls, distended perivascular spaces. Diapedesis. Glial reaction near the vessels. Pigment.	Satellitosis. Neuronophagia. Slight proliferation near the vessels.	Cytoarchitecture well preserved, only small scattered acellular areas. Focal chromatolysis. Both phenomena mostly in the vicinity of the vessels.	0	Ditto. Mostly reversible changes. The pigment at least partly fatty products of degeneration, frequently incorporated in large phagocytic elements. Sometimes serial sectioning.
Immersion	Nissl, htx-eosin, Niemer.	0	Vascular or capillary engorgement, perivascular spaces slightly distended. Scattered subpial hæmorrhages.	0	0	Same changes as in the treated animals.	The material examined independently by three pathologists, who were unaware of which cats were treated and which were controls. — The changes observed attributed to anesthesia & post-mortem artefacts.
Perfusion by isotonic solutions	Buffered thionine, Weil.	0	0	0	0	0	Perfusion fixation may diminish post-mortem artefacts. "Failure to recognize post-mortem artefacts may explain much of the cytopathologic alteration reported by other investigators who have attributed their observations to ECT".