THE MODE OF ACTION OF ELECTRO-CONVULSIVE THERAPY.

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How electro-convulsive therapy (E.C.T.) acts, what is the chain of cause and effect from the electrical application to the clinical improvement, are questions whose answers are as uncertain as ever, in spite of the importance of such knowledge to psychiatry and in spite of the researches of nearly twenty years. The experiments reported in this paper are an attempt to elucidate the links of one possible chain. What are the possibilities ? I shall review them briefly, restricting the review to the physiological and biochemical aspects and omitting any reference to the psychological or psycho-analytical.

The review is made difficult, and the analysis of the experimental evidence complicated, by the fact that the variables are inter-related in a complex pattern. They form a dynamic whole such that a disturbance at any point causes changes sooner or later at almost all other points. The chief variables and their main direct effects are shown in Fig. 1, which, though showing only the essentials, shows clearly the complexity of the interactions. I shall consider the variables at first in pairs.

The evidence that E.C.T. affects the hypothalamus is mostly indirect, though the uniformity with which the hypothalamus reacts to almost every form of stress leaves little doubt that E.C.T. will act similarly (Cheng *et al.*, 1949 ; Farrell and McCann, 1952 ; Fortier, 1951 ; Long and Fry, 1945 ; Selye, 1946, 1952). The electric current is certainly likely to excite the hypothalamus directly, for both Lorimer *et al.* (1949) and Hayes (1950) have shown that much of the current passes through the basal structures, where it would stimulate the hypothalamus both directly and through the stimulation of such structures as the medial forebrain bundle, the thalamo-hypothalamic fibres (with their relay at the periventricular nuclei), the fornix, the *stria terminalis*, and the pallido-hypothalamic fibres, structures whose stimulation would necessarily lead to stimulation of the hypothalamus. There is no doubt that an electrically-induced convulsion (" E.I.C." to distinguish it from the therapy) can affect the structures of the diencephalon, for Wikler and Frank (1948) have shown, in the decorticated cat, that E.I.C. abolishes the diencephalic reflexes of righting, licking and sham rage. Roth (1952), too, has found evidence that E.C.T. has an effect on the hypothalamus.

Evidence that E.I.C. affects the pituitary has been obtained mostly by observing the effects on the pituitary's targets, for direct evidence of the pituitary's reaction is hardly to be obtained. There is little doubt that the pituitary can be affected directly in its posterior part, for Altschule *et al.* (1948) showed how E.C.T. evokes several signs of its activity, and Harris (1948) confirmed the action in the rabbit. He also showed, with Markee (1946), that prolonged electrical stimulation to the rabbit's hypothalamus induced ovulation, whereas direct stimulation of the pituitary failed to induce it—clear evidence that so far as the secretion of luteinizing hormone is concerned, the hypothalamus has no nerve-endings in the pituitary but must act in some other way. Whether this other way could be a hormone of the type considered by the Scharrers (1937) is still unknown.

The relation between E.C.T. and the secretion of adrenal steroids (Fig. 1) is both simpler and clearer, for the secretion of these substances can be observed almost directly. Thus E.C.T. was found by Ashby (1949) to cause a brisk outpouring of steroids in the first few days of treatment ; and this was confirmed in another study (Ashby, 1952*a*) of a periodic catatonic who was treated with E.C.T. at intervals over five months. Other workers' studies have relied on less direct evidence of adrenocortical activity. Thus Hoagland *et al.* (1950) used a variety of indirect

tests when they showed that E.C.T., even when given in subconvulsive doses, caused an adrenocortical activity distinctly larger than that evoked by 25 mgm. of A.C.T.H. (Armour La-1-A). Similarly the numbers of circulating eosinophiles and lymphocytes have been used to show, by their fall after E.C.T., that adrenal steroids have been secreted (Altschule, *et al.*, 1949*a*, *b* ; Mikkelsen and Hutchens, 1948).

The effect of E.C.T. on the cerebral metabolism (Fig. 1) is probably complex, for a variety of modes may combine to give the final pattern. Klein and Olsen (1947) have demonstrated some of the effects, showing that the amounts of glucose, glycogen, phosphocreatine, and ATP are decreased after electrical stimulation and after the giving of convulsant drugs ; while lactic acid, ADP and inorganic phosphate are increased. Their results have been confirmed and extended by Dawson and Richter (1950), who used liquid air to ensure almost instantaneous immobilization of the metabolites, so that the biochemical changes could be followed from within a second or two of the giving of the shock. That the events in the first few seconds may be important is also suggested by the work of McIlwain *et al.* (1951), who showed that when the rat brain was stimulated *in vivo* there occurs, in the first few seconds, an increase in O_2-respiration, in glucose utilization and in lactic acid formation much greater than occurs when similar stimulation is given to slices *in vitro*.

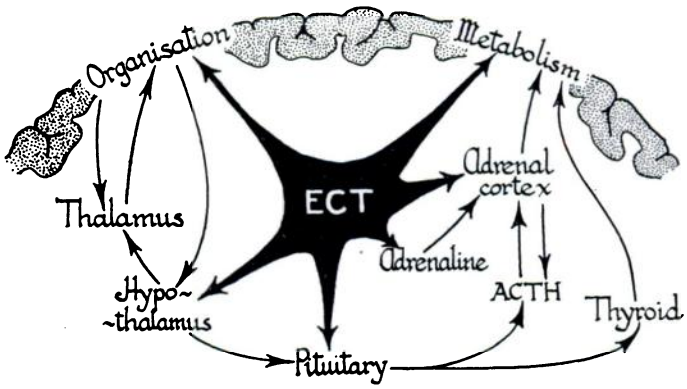

FIG. 1.—The most important variables affected by E.C.T., and their main direct effects, each of which is represented by an arrow.

Another route by which E.C.T. may affect the cerebral metabolism is by way of its effect on the organism's sensitivity to adrenaline and insulin. Gellhorn and Safford (1948), for instance, found that after E.I.C. rats became more sensitive to adrenaline, and this increase was confirmed by Funkenstein *et al.* (1948) in persons both normal and psychotic. Prokop (1947), however, obtained the contrary result, and Ashby (1952*a*), in a prolonged study of a patient who was already markedly insensitive to adrenaline, found E.C.T. to have little effect on the insensitivity. In this connection it should be appreciated that the system of Fig. 1 has several interlacing feedbacks and is therefore reactive, responding to any action of environment or experimenter with opposition. Thus, while the secretion of adrenal steroids is largely determined by the amount of A.C.T.H. in the blood, and partly by the amount of adrenaline (Recant *et al.*, 1950 ; Farrell and McCann, 1952), the release of A.C.T.H. from the pituitary is itself controlled, to some degree, by the amount of circulating steroid (Sayers and Sayers, 1947). Similarly the giving of E.C.T. has sometimes made patients less sensitive to A.C.T.H. (Altschule *et al.*, 1950), though those who were more sensitive showed a better therapeutic response to E.C.T. (Hoagland *et al.*, 1950). There is, therefore, some evidence that E.C.T. acts on the pituitary, causing it, perhaps among other actions, to secrete A.C.T.H. The next question is whether such a secretion might be responsible for the therapeutic effects. Various studies have been made, such as that of Clow and Prout (1946), on the psychiatric effects of such accidents and illnesses as are likely to evoke a secretion of A.C.T.H. ; but more direct evidence has become available since the hormone was

isolated. When A.C.T.H. was given to normal subjects (Mason *et al.*, 1948 ; Thorn *et al.*, 1948), in doses sufficient to cause well-marked physical changes, the mental changes have been slight or absent, the only sign shown by Mason's subject, for instance, being that he became " listless and apathetic." When patients with physical illnesses (often rheumatoid arthritis) were treated with A.C.T.H., a wide variety of psychic reactions were observed, with euphoria as perhaps the commonest response (Sprague, 1951). Other workers, however, have rightly emphasized the stimulating effect of the suggestion that a crippling illness was to be ended, and have considered that the responses were simply manifestations of the patients' previous personalities (Brody, 1952 ; Rome and Braceland, 1950).

When A.C.T.H. was given to psychotic patients as treatment for their psychoses the results have been, in general, disappointing. Altschule and his colleagues (1949*a*, 1950) have reported three cases, all melancholics, who received A.C.T.H. in large doses without improvement, and who then received E.C.T. and recovered. Cleghorn *et al.* (1950) and Smith (1950) both tried it in mania and in melancholia ; their patients showed either no improvement or one that was doubtful and transitory. Nor does the schizophrenic respond more favourably : it seemed for a time as if the treatment proposed by Lewin and Wassen (1949) offered prospects of success, but Rees and King (1951), in a carefully controlled study, found no evidence that it gave any benefit. This treatment used D.O.C.A. : even when the more physiological A.C.T.H. was used the results were no better, for the responses found by Glaser and Hoch (1951) and by Hoagland *et al.* (1950) were, so far as the psychological symptoms were concerned, few and slight. Cortisone proved equally inefficacious (Cohn *et al.*, 1951 ; Rees and King, 1952).

When we turn to the action of E.C.T. on the cerebral cortex, we find the facts to be suggestive but not yet fully understood. If E.C.T. acts on the pituitary and adrenal it is likely to affect the cerebral metabolism, for pituitary extracts and adrenal steroids are known to have many effects on enzymes, such as hexokinase (Colowick *et al.*, 1947 ; Stadie *et al.*, 1950), phosphatase (Kochakian and Vail, 1944), cholinesterase (Hawkins and Nishikawara, 1949 ; Torda and Wolff, 1952), arginase (Folley and Greenbaum, 1946), cytochromes (Tipton, 1944), and lipoids (Campbell and Lucas, 1951)—all powerful factors in the cerebral metabolism.

One factor remains to be discussed. There is a possibility that E.C.T. may have a direct effect on the cortical machinery, not in its biochemical but in its cybernetic components, in its organization. Modern physiology regards the cerebral cortex as functioning not as a simple machine of a few determinate parts, but as a very great network in whose properties the statistical element must inevitably be prominent. Lashley (1952), for instance, uses the following words :

> " Even the simplest bit of behavior requires the integrated action of millions of neurons ; . . . I have come to believe that almost every nerve cell in the cerebral cortex may be excited in every activity. . . . Differential behavior is determined by the combinations of cells acting together rather than by cells which participate only in particular bits of behavior. The same neurons which maintain the memory traces and participate in the revival of a memory are also involved, in different combinations, in thousands of other memories and acts."

The study of such statistical systems of neurons has only just commenced, but already some facts are known. It has been shown (Ashby, 1952*b*) that one fundamental property that such systems will tend to show is that their responses, other things being equal, will tend to diminish. When the stimulus is repeated monotonously, the phenomenon is well known under the name of " habituation." We can also recognize, in everyday experience, a tendency for what is at first interesting and evocative to become later boring and uninspiring. Whether the extreme unresponsiveness of melancholia is really an exaggeration of this process is unknown, but the possibility deserves consideration. What makes the possibility specially interesting is that the theory of such statistical systems makes it quite clear that any complex network that has progressed to a non-responding state can, in general, be made responsive again by administering to it any large and random disturbance. The theory also makes clear that such a disturbance will necessarily disturb severely the system's memory : the parallel with E.C.T.'s effect on memory is obvious. Whether, however, E.C.T. acts in essentially this way is a question for the future.

These are the main relations between the variables shown in Fig. 1. In this paper are reported some experiments designed to explore in more detail the actions—

E.C.T.—→adenohypophysis—→adrenal cortex.

Experiments.

In these experiments we used the rat (Wistar strain, closely inbred) and attempted to investigate the extent to which the adenohypophyseal-adrenocortical system was activated by E.I.C. For this purpose we needed an indication of the amount of A.C.T.H. secreted. Direct assay of its amount in the blood was, at the time of these experiments, not possible, though the work of Farrell and McCann (1952) has recently suggested that such an assay may be possible eventually. Practically our choice was restricted to estimating, in the adrenals of the rat that had received the E.I.C., the falls of either the ascorbic acid or of the cholesterol (Sayers and Sayers, 1947). For several reasons, including the fact that the rat can synthesize ascorbic acid without difficulty (Najjar and Barrett, 1945), we preferred to assay the cholesterol. After some preliminary studies the following method was selected.

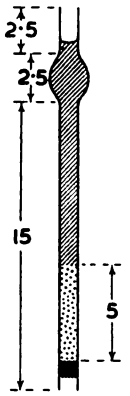

Fig. 2.

For the administration of the E.I.C., the ears were well cleaned with spirit, wetted with saline, and held by " crocodile " clips with flattened surfaces. (The ears were used in preference to flat electrodes on the head, for the ears force the current to enter the head at a place that is invariant anatomically.) Sinusoidal current at 50 c.p.s. was applied usually at 100 V.; its duration was controlled by a mechanical switch with a range from 0·1 to 1·0 seconds. (As will be shown in Experiment 5, these conditions, either of voltage or of timing, do not seem to be critical; neither does that of the site of the electrodes, for Lorimer *et al.* (1949) and Hayes (1950) showed that the current tended, wherever started, to go diffusely through the brain, favoring slightly the basal structures.) At the appropriate time the animal was killed by neck-break, both adrenals at once removed, carefully freed from fat, weighed to 0·1 mgm. on a torsion balance, and ground to a smooth powder in 1 gm. of anhydrous sodium sulphate. (Tests showed that in this condition the cholesterol was stable practically indefinitely.)

The cholesterol was extracted by chloroform, and as our method had some novel features and proved rapid, simple, and accurate, it will be described in detail. Extraction tubes were made to the size and shape shown in Fig. 2, which shows the extraction in progress. The lower end was stopped with a small plug of grease-free cotton-wool (shown heavily shaded), which retained the sodium sulphate-adrenal powder (stippled). 5 ml. of chloroform was then added on top (light shading) and the cholesterol eluted.

Experiment 1.

Before the method was adopted, tests showed that the extraction was rapid and complete. By collecting the eluate in successive portions it was found repeatedly

that the first half ml. contained at least 95 per cent. of the cholesterol, and that the second half ml. contained the remainder. No trace could be found in the subsequent portions, tested at each half ml.

The eluate was adjusted to an exact volume, an aliquot taken when the rats were female (for their adrenals contained about twice the amount contained by the males'), the colour developed with acetic anhydride and sulphuric acid, and the estimation made photoelectrically. Both " blank " and " standard " tubes always accompanied each batch.

After these preliminaries there arises the question of how to measure the cholesterol's fall: what shall serve as control? Obviously, the ideal would be the same gland before the convulsion, but this, of course, was impossible, for the first analysis necessitated its destruction. We therefore considered the possibility of using the same animal's other adrenal, one being removed before and the other after the convulsion. (The fact that the left gland contains, on the average, more cholesterol by 9 per cent. must be borne in mind here.) We therefore explored the possibility of using the rats in pairs, removing the right adrenal from one and the left from the other before convulsion, administering the E.I.C., and then, at the appropriate time later, removing the remaining adrenals, left and right, thus providing

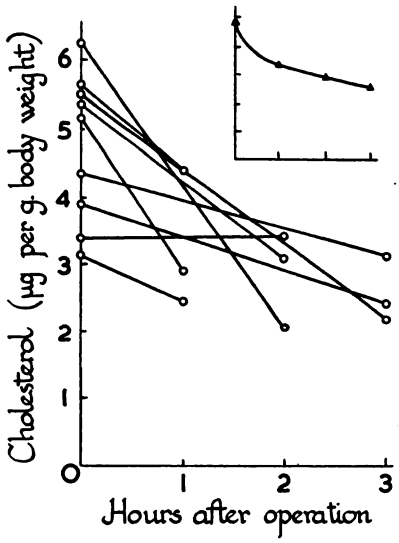

FIG. 3.—Changes in the cholesterol content of the remaining adrenal after unilateral adrenalectomy. The small graph inset shows the changes of the average. Immature rats.

a pair of observations that were balanced for laterality. The method, however, requires that each animal be anaesthetized and operated on prior to the E.I.C., and it is well known (Venning, *et al.*, 1944; Talbot *et al.*, 1947) that such treatment is likely in itself to cause a secretion of A.C.T.H. and a lowering of the adrenal cholesterol. As, however, the method would be valuable if it could be made to succeed, its possibility was tested.

Experiment 2a.

Eighteen rats were selected as nine carefully equalized pairs. Each pair was anaesthetized with ether and the right adrenal taken from one and the left from the other by the lumbar route. No convulsion was given. At 1, 2, or 3 hours after the operation (three pairs at each time) the rats were killed and the remaining glands at once removed. All were assayed for cholesterol in the manner described, and the results were examined for any fall in cholesterol due to the disturbing effect of the anaesthetic and operation. The results are shown in Fig. 3, in which each circle-ended line shows, at its left-hand end, the amount of cholesterol at the operation

and, at its right-hand end, the amount in the contralateral glands of the same two rats at the indicated time later. The figure shows that all pairs but one lost cholesterol from their adrenals in the first few hours after the operation.

These rats were immature, with weights between 43 and 49 gm., for we were interested at the time in their use; there remained the possibility that adult rats might yet prove usable, so a further experiment was performed.

Experiment 2b.

Six batches, each of six rats closely matched for family, age, and weight were taken, three of the batches containing males only and three females only. Each batch was treated in the same way: the six rats were divided into three pairs; each pair was adrenalectomized as before, and at 1, 2, or 3 hours after operation the remaining adrenals were removed; all were assayed for cholesterol. For expressing the results the scale of Fig. 3 was not satisfactory, for the females, as was expected, gave about twice the quantity given by the males. Each batch, however, being uniform in sex, is comparable within itself, so the results were reduced, for Fig. 4,

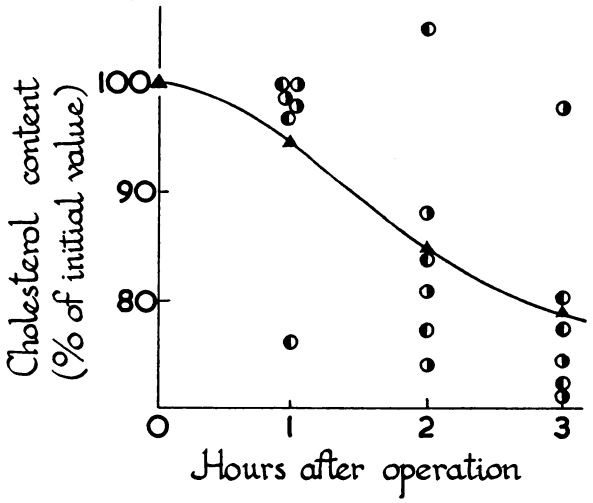

FIG. 4.—Changes in the cholesterol content of the remaining adrenal after unilateral adrenalectomy. Mature rats. Each circle is the average of two rats: ◑, female; ◐, male; ▲, average of 12 rats.

to the common scale that takes the initial values as 100 arbitrary units. The sexes have been indicated in the figure: inspection shows no particular difference between the sexes in their *relative* responses. The trend, shown by the continuous line through the averages, is clearly downwards. The two experiments (*2a* and *2b*) thus show that the operation of adrenalectomy, with its accompanying anaesthesia, is a significant diminisher of the adrenal cholesterol.

We were thus forced to use, as control for the test rat, another rat. This allows the variance between rats to come in as experimental error; but we could find no way of avoiding this. The effect was, however, minimized by arranging that the test rat and its control should always be selected to be closely similar, and by ensuring that all averages and other statistics were based on a generous number of animals.

Our next step was to find at what time after E.I.C. the adrenal cholesterol became minimal, for estimations made at that time would not only be maximally sensitive but would also be least affected by disturbances which shifted the minimum.

Experiment 3.

As a preliminary exploration, rats, adult and of both sexes, were given an E.I.C. and at various times later, from 0 to 24 hours, were killed and the adrenals assayed, right and left separately. The amounts in the two glands were then added, and the

result for each animal expressed as μg. of cholesterol both per gm. of body weight and per mgm. of adrenal weight. The two forms behaved similarly with time, and the results for the former are shown in Fig. 5, in which the time-scale has been distributed non-linearly for clarity of display. The two sexes have been indicated separately to show any difference of trend. These differ in no essential, and the results for the thirty rats show that, to a first approximation, the minimum does not occur until 4 to 5 hours after the convulsion, and that it lasts for some hours.

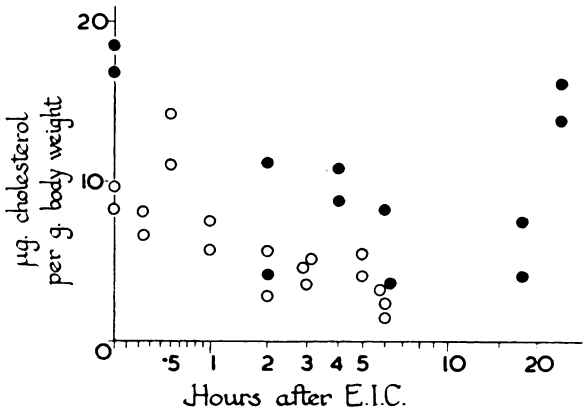

FIG. 5.—Changes in adrenal cholesterol after E.I.C. Adult rats; ●, female; ○, male. (The abscissae are graduated as $\log_{10}(1 + \text{time})$ in order to space the results more suitably.)

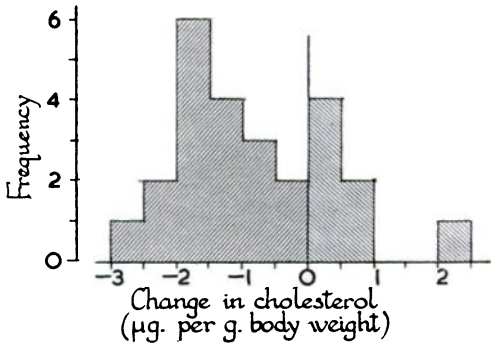

FIG. 6.—Changes in adrenal cholesterol four hours after E.I.C. A shift to the left implies that the amount fell.

This experiment was sufficient to indicate the appropriate time for the removal of the adrenals after E.I.C., but it was intended only as a preliminary survey, so we arranged a further experiment to show specifically the effect of E.I.C. on the adrenal cholesterol.

Experiment 4.

Twenty-five pairs of rats, all male and adult, were accurately matched and the two of each pair were treated similarly in almost every way, each rat being taken through the routine for the giving of the shock (100 V. for 0·1 second), but to only one of the pair was the shock actually given. Four hours later both were killed and the cholesterol assayed as usual. From each pair was found the quantity "cholesterol in the convulsed less the cholesterol in the unconvulsed rat" (both expressed as μgm. in both adrenals per gm. of body weight). Taking the difference in this way makes the result, of course, independent of variations between pair and pair, such for instance, as were bound to occur between one day and another; for the whole experiment, with its fifty rats, was necessarily spread over a number of days.

The changes following the convulsion are shown in Fig. 6, which shows the tendency for the change to be negative, i.e., for the adrenal cholesterol to *fall* after E.I.C. The fall, as an average, was from 5·84 (μgm. cholesterol in both adrenals per gm. body weight) to 4·97, a fall of 15 per cent. The trend, though not showing in every pair, is statistically significant, for t is 3·63 and P is less than 0·002.

Although these results showed that E.I.C. would cause the adrenal to lose cholesterol, the loss was not large, and we decided to test whether a more prolonged shock would give a larger effect. The experiment was also of interest in view of the variety of opinions that have been expressed about the amount of shock that is optimal therapeutically.

Experiment 5.

Adult male rats were associated into accurately matched triples, and each of the triple was treated similarly except that one had a shock (at 100 V.) of 0·1 second, one had a shock of 1·0 second, and one had the electrodes placed but no shock. Four hours later the animals were killed by neck-break, and the adrenals, after rapid weighing to the nearest 0·1 mgm., were assayed for cholesterol. The triples were used because there were far too many animals to be taken through as a single batch; in fact, the experiment occupied some weeks. The three rats of a triple, however, could be taken through in one day and almost simultaneously, so they provided accurate controls for each other. The comparisons (i.e., differences) between rats in the same triple (though not the absolute values) could thus legitimately be combined and compared, being free from those effects that make one triple uniformly different from another. As the control for one rat's response was simply another rat, the comparatively large number of 48 triples (144 rats) was used, so that the standard error of the final averages should be sufficiently small.

When the results were examined, it was found that the variance in the different classes did not seem to be homogeneous, those with more cholesterol tending to have a larger variance than those with less; evidently, as often happens in biological material, it was the coefficient of variation, rather than the variance, that tended to be uniform. The uniformity required for the analysis of variance was therefore obtained by a transformation of the weights of cholesterol to their logarithms. This transformation was used for all the data from this stage onwards. It does not really add any complication, either conceptual or mathematical; for it amounts, when we compare "test" and "control," to our finding by how much per cent. the one exceeds the other instead of by how much in weight.

The experiment was also planned to provide information on another question. From the inception of the work there had been some doubt about the best way of expressing the results of the assay for cholesterol, which could, for instance, have been expressed "per rat," or "per gm. of body weight," or "per mgm. of adrenal weight," and so on. Some experimenters still decide such questions by selecting one of such forms arbitrarily, probably because they are not acquainted with the statistical principles involved; but there is now no doubt that the best method is that the experiment should be arranged so that the results themselves can declare which form is best. In our particular case previous testing had shown that results given as "μgm. per gm. body weight" had no advantage over the simple "μgm. per rat." Also, in most of the experiments each pair or triple, which alone provided the basic comparisons, was uniform for body weight, the members having been selected to this end. "Correction" for body weight was therefore unnecessary. The status of "adrenal weight" was, however, less clear. The adrenal weight not being known till after death, the selection of rats for adrenal uniformity was not possible, so some correction seemed called for; but whether the correction should be a simple weight-for-weight adjustment, or any other, was not known. Fisher (1935), however, has shown that the proper method is to use the variable (e.g., adrenal weight) as concomitant, and then, by an analysis of the covariance, to deduce from the experiment itself what correction, if any, is appropriate. This method was used in this and the next experiment (Expts. 5 and 6). Experiment 5 showed, if I may anticipate its results, that the gland's weight, when all other sources of variation had been allowed for, was not significantly correlated with its content of cholesterol, the actual value being $-0{\cdot}088$. (Its square was 0·0077, which means that less than 1 per cent. of the variance in cholesterol content could be associated with the variance in weight.) Evidently the variation in weight was due mostly to water, or to some substance or tissue not causing a variation in the

content of cholesterol. What little correlation there was, was negative, showing how wrong would have been the application of the commonly, and thoughtlessly, applied " correction for weight." From this point onwards, therefore, we used, as main variable, the logarithm, to the base 10, of the total weight of cholesterol.

The analysis of variance of Experiment 5's main results is shown in Table I. The sources of variation are : (*a*) that between Triples, which includes all the sources

TABLE I.—*Expt.* 5 : *Analysis of the Variance of " Log (Cholesterol)."*

Variance.	D.F.	Sum of sq.	Mean sq.	*F.*	*P.*
Triples . . .	47	1·01259	$21·54 \times 10^{-3}$	2·51	<0·001
Durations . . .	2	0·10323	51·61 ,,	6·00	<0·01
Residual . . .	94	0·80794	8·60 ,,	—	—
Total . . .	143	1·92376	—	—	—

to which the triples were exposed differentially, e.g., that two triples differed in the day on which they were experimented on ; (*b*) that between Durations, which includes the different effects of giving no shock, a shock of 0·1 second, or a shock of 1·0 second—the main interest of the experiment ; and (*c*) Residual, all the other factors which might make one member of a triple differ from the other two members. The right-hand column shows that the variation due to Triples was highly significant, showing that the carrying of the experiment through in small uniform batches (each of three rats), each with its own internal controls, was well worth while as an eliminator of errors. Further, the effects of the different shocks is clearly significant, with P less than 0·01.

The analysis shown, however, while essential as a preliminary, does not yet show exactly what we are interested in. The two degrees of freedom for " Duration " consist both of the effect of giving an E.I.C. (as contrasted with not giving one) and also of the effect of prolonging the current-flow from 0·1 to 1·0 seconds. These two effects, each a single degree of freedom, can be separated. Thus, if x, y, and z are the results in one triple of no shock, shock of 0·1 sec., and shock of 1·0 secs. respectively, then the linear function, $u = 2x - y - z$, will measure the effect of the shock, and the second linear function, $v = y - z$, which is orthogonal to the first, will measure the effect of the prolongation. These quantities were calculated for the 48 triples, and their sums of squares found to be, for u, 0·0950, and for v, 0·0082. (The fact that they sum to 0·1032 checks both the reasoning and the arithmetic.) Of these, u is significant with P equal to 0·001, while v is quite insignificant. Clearly the important factor is the giving, or not giving, of E.I.C. : how long the current passes makes no detectable difference to the adrenal cholesterol.

This last deduction was of some interest, for the actual average contents (as logarithm) in the three classes were in the ratios : no shock : 100, 0·1 sec. shock : 65, 1·0 sec. shock : 75, and it seemed possible that the increased shock might actually cause less response, perhaps by some paralysing effect. The tests of significance show, however, that there is no evidence for a reversal.

To sum up the results of this experiment. The main fact is that the degree of freedom u, the comparison of the shocked with the unshocked rats, is significant with P equal to 0·001. The experiment thus demonstrates decisively that an E.I.C. lowers the cholesterol content of the rat adrenal. Minor results are that the duration of the electric current, within the range 0·1–1·0 seconds, makes little difference.

THE EFFECT OF HYPOPHYSECTOMY.

Only one cause is known to be efficacious in the lowering of adrenal cholesterol : a secretion of A.C.T.H. by the adenohypophysis. In order to test directly whether this really was the cause of the fall, a new set of experiments was devised on the basis that if such a secretion was the cause of the fall, then the fall should be prevented by a previous hypophysectomy.

Before the new experiments were commenced, it was clear that careful consideration would have to be given to their design. Thus, if we take it as axiomatic that the new experiments must include a complete set of controls, at least four groups of animals will be required. First, to demonstrate that the E.I.C. *is* having an effect, there will have to be two groups, which can be called A_c (the convulsed) and A_0 (the unconvulsed) ; then these two groups will have to be contrasted, in their adrenal cholesterol contents, with two hypophysectomized groups, B_c (the con-

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vulsed) and B_0 (the unconvulsed). Then if the A's do show the effect of E.I.C. and the B's do not, the effect of the hypophysectomy will have been demonstrated. The logical structure thus demands, apart from replication, four animals to the unit—one each of A_c, A_0, B_c, B_0—and this basic structure must be retained through any further complications.

In technical language the experiment tests for an interaction. In the usual notation (Fisher, 1935; Yates, 1937), if H is the factor that can take the two values H_0 and H_1 representing "normal" and "hypophysectomized" respectively, and if C can take the two values C_0 and C_1 representing "not convulsed" and "convulsed" respectively, so that any particular animal's condition is represented by giving values to i and j in C_iH_j, then the interaction will be measured by the linear function

$$C_1H_0 - C_0H_0 - C_1H_1 + C_0H_1;$$

for this compares the effect of convulsion in the normal animals, $(C_1 - C_0)H_0$, with its effect in the hypophysectomized, $(C_1 - C_0)H_1$. The null hypothesis then is that the values of this linear function, each value derived from the adrenal cholesterols of four rats, do not, on the average, differ significantly from zero.

In addition to the factors C and H, and their interaction CH, we wished to take into account the possibility that the feeding of the animals in the few hours before the experiment might be an important factor, partly because of our own chance observations, and partly because Henriques *et al.* (1949) had shown that rats on high protein diet seemed to be particularly sensitive to lyophilized anterior pituitary extract. (That they were observing chiefly the action of the A.C.T.H. in the extract is clear from the fact that they were observing its power to deplete the adrenals of ascorbic acid.) As our experiment would in any case have to contain much replication (to lessen sampling irregularities), and as there was no prior knowledge of whether it was better to feed or to starve, we decided that variation of this factor should be introduced deliberately. In addition, as there was some uncertainty about the best time for examining the adrenals (the number of hours after the shock) and as the other factors, e.g., hypophysectomy, might interact with this, rendering the previous estimate of Expt. 3 inaccurate, variation of this factor seemed advisable. We decided therefore to investigate all sixteen combinations of the four factors, each at two values:

C: Unconvulsed (C_0)/convulsed (C_1) animals;
H: Intact (H_0)/hypophysectomized (H_1) animals;
F: Those fed *ad lib* throughout (F_0)/those starved overnight and until operation (F_1);
T: Those killed three hours after convulsion (T_0)/those killed five hours after convulsion (T_1).

(For convenience of explanation, the sixteen animals necessary for the sixteen combinations will be referred to throughout as a "set.")

This arrangement, though giving a thorough exploration of the relevant factors, raised difficulties in that it required that sixteen animals should be taken through the experimental procedures together. With our resources this number was, in our opinion, too large to be treated uniformly; in particular, the set was too large for all the animals to be taken through the procedures in the same day; and we were of the opinion that, in work such as this, it is desirable that comparisons should be made only between animals that were treated within the same hour. The question therefore arose whether the modern technique of confounding could not be used advantageously. In fact, using the notation above, we were interested primarily in the interaction CH, and secondarily in the higher interactions CHF, CHT, and $CHFT$ (for these were needed to interpret the significance of CH); but our interest in some of the other factors and interactions was slight, so they could advantageously be confounded, preferably in the "partial" form, so that information on these factors and interactions was not lost totally. On considering the circumstances it was considered that a good balance would be given by using sixty-four rats in four sets of sixteen, and by confounding:

in set 1: H, F, and HF
,, 2: C, T, and CT
,, 3: T, CF, and CFT
,, 4: H, FT, and HFT.

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(This arrangement obeys the mathematical necessity that, if P and Q are confounded their interaction PQ shall also be confounded.) It gave a much improved experimental design, for each batch that must be kept homogeneous was reduced from sixteen rats to four, a number that could be taken through in one day with satisfactory uniformity. (I shall, from here on, reserve the word " batch " for such a four). Its disadvantages were, that the experiment had to be carried through in accordance with a somewhat complex schedule, a typical batch consisting of the four treatments $C_1H_0F_1T_1$, $C_1H_0F_1T_0$, $C_0H_0F_1T_1$, $C_0H_0F_1T_0$, and that the subsequent statistical analyses were more complex than usual; these however were trivial when balanced against the improvement in experimental accuracy.

One minor point calls for comment. Strictly, the experiment contains " dummy " comparisons, for a removal of the adrenals at a nominal 3 hours after " no convulsion " is not factually different from a removal at 5 hours after the same null operation. Had there been no confounding, these comparisons would have been isolated in the usual way and added to the residual variance; but the presence of confounding, especially as it is partial, makes the analysis very complex; and as the effect is in any case trifling, it seemed simpler to ignore this complication, which occurs in only a fraction of the comparisons.

So much for the design. With regard to the operative details, the hypophysectomies were performed by the pharyngeal route, under ether anaesthesia, with the technique of Smith (1926) as basis. When each rat was eventually killed, the pituitary fossa was examined under the dissecting microscope: if any pituitary tissue was found remaining, that batch of four rats was discarded and the batch repeated.

Experiment 6a.

This experiment, on sixty-four rats, was completed, but no great confidence was felt in the results, for the operative and other techniques had but recently been learned. A repetition of it (Exp. 6*b*) was therefore performed, and then, when it was clear that the techniques were fully mastered, a third (Exp. 6*c*).

The results of each of these experiments (6*a*, *b* and *c*) were examined in full statistical detail by the analysis of their variance and covariance, with " adrenal weight " as concomitant. Its 63 degrees of freedom were analysed into 16 single degrees of freedom corresponding to the main effects and their interactions, which includes the focus of interest, CH; 15 degrees of freedom, which need not be further subdivided, between the batches; and 33 residual. The discussion of the results is best preceded by the elimination of some minor topics.

" Adrenal weight " can be dismissed at once, for the three parts of Expts. 6 showed, as did Expt. 5, that it contributed no useful information towards the main effects that we were interested in. The three partial correlations, all other variables and factors being held constant, were + 0·332, − 0·029, and − 0·231. As the average value is + 0·024, markedly less than its standard error, there is no evidence of appreciable correlation. Evidently the factors that varied the adrenal weights did not affect their content of cholesterol.

The factors F and T were not only insignificant in themselves but proved to have no significant effect on the CH interaction; for the interactions CHF, CHT, and $CHFT$, even when tested over all the 196 rats, were insignificant. These factors need not, therefore, be considered further.

A possible source of error is that the hypophysectomy may so cause the adrenal to atrophy, or its cholesterol content to fall, that the adrenal becomes unresponsive to A.C.T.H. The average changes after hypophysectomy, with their standard errors, were found from the results and are shown in Table II (in arbitrary units). It will

TABLE II.—*Effects of Hypophysectomy.*

		On adrenal weight.	On cholesterol content.
Expt.	6*a*	− 0·22 ± 0·46	+ 3·8 ± 2·0
	6*b*	+ 0·15 ± 0·54	+ 7·2 ± 2·1
	6*c*	+ 0·60 ± 0·61	+ 5·5 ± 2·7

be seen that the effect of hypophysectomy on the cholesterol content is to increase it significantly, the increase being probably due to the lack of circulating A.C.T.H. and to the consequently decreased mobilization. Its effect on adrenal weight is equivocal, but at least there is no marked fall. If therefore E.I.C. fails to cause a fall of cholesterol in the hypophysectomized animals, the failure cannot be due to any lack of cholesterol in their glands.

One other preliminary question remains. At one time it was thought that the rats' body weights might have some relation to their response to E.I.C. The protocols of Expts. 4 and 6 were therefore re-examined, for each rat's body weight was always recorded. The product-moment correlation between weight and response was found in each homogeneous group, and the correlations examined as a set. No bias was found, so the facts showed no reason for thinking that body weight played any significant part.

We can now turn to the main question that Experiments 6*a*, *b*, and *c* were intended to answer: the effect of hypophysectomy on the reaction to E.I.C. The results for the three experiments are shown, first by the analysis of variance in Table III, and then in Table IV, which shows the average values so that the sizes and directions of the changes can be seen.

TABLE III—*Expt.* 6: *Analysis of the Variance of "Log (Cholesterol)."*

Variance or factor.	D.F.	Sum of sq.	Mean sq.	*F.*	*P.*
C	1	136 × 10⁻⁶	136 × 10⁻⁶	—	—
H	1	288204 ,,	288204 ,,	16·6	<0·001
CH	1	58451 ,,	58451 ,,	3·38	>0·05
F	1	11201 ,,	11201 ,,	—	—
CF	1	36736 ,,	36736 ,,	2·12	>0·05
HF	1	1 ,,	1 ,,	—	—
CHF	1	6188 ,,	6188 ,,	—	—
T	1	17334 ,,	17334 ,,	—	—
CT	1	25676 ,,	25676 ,,	1·48	>0·2
HT	1	20626 ,,	20626 ,,	1·20	>0·05
CHT	1	278 ,,	278 ,,	—	—
FT	1	27792 ,,	27792 ,,	1·60	>0·05
CFT	1	47669 ,,	47669 ,,	2·76	>0·05
HFT	1	1 ,,	1 ,,	—	—
CHFT	1	713 ,,	713 ,,	—	—
Sets	11	754389 ,,	68581 ,,	—	—
Batches	36	1196993 ,,	33250 ,,	1·92	<0·01
Residual	129	2232019 ,,	17302 ,,	—	—
Total	191	4724408 × 10⁻⁶	—	—	—

TABLE IV.—*Average Values of Log (Cholesterol) in Entire* (H_0), *Hypophysectomized* (H_1), *Unconvulsed* (C_0), *and Convulsed* (C_1) *Rats.*

	Experiment.							
	6*a*.		6*b*.		6*c*.		All 6.	
	H_0.	H_1.	H_0.	H_1.	H_0.	H_1.	H_0.	H_1.
Convulsion C_0	·313	·321	·245	·299	·236	·286	·265	·301
Convulsion C_1	·277	·345	·211	·297	·252	·314	·247	·319

The average over all the three parts of Experiment 6 is shown in the two right-hand columns of Table IV. Each quantity is not a direct measurement but a reconstruction from somewhat complex comparisons, involving a variety of balanced controls. In these four values something of the expected result is shown; for in the unoperated animals (H_0) the effect of a convulsion is to make the content of cholesterol (on the logarithmic scale) fall from 0·265 to 0·247, while in the hypophysectomized (H_1) no such fall occurs (there is actually some increase from 0·301 to 0·319). Neither of these effects, however, can claim statistical significance; for as the four quantities are each based on 48 rats, and as the average variance per rat (Table III) is 0·0173, so the variance of each quantity (under "All 6") will be 0·0173/48, i.e., 0·00036, and its standard error 0·019. The results thus show changes in the direction expected, but not amounting to statistical significance. It seems clear, therefore, that though hypophysectomy lessens, or counteracts, the effect of convulsion, the factors responsible need further elucidation.

SUMMARY.

The paper describes experiments intended to elucidate the mode of action of electro-convulsive therapy. In particular it reports how convulsion, in the rat, affects the adenohypophyseal-adrenocortical system.

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The experiments showed that:

(1) Anaesthesia and unilateral adrenalectomy leads after a few hours to a fall of the cholesterol content in the other adrenal.

(2) The fall occurs in both mature and immature animals.

(3) The weights of the adrenal glands and of the whole body were found, throughout the experiments, not to be correlated with the gland's cholesterol content.

(4) An electrically induced convulsion causes the adrenal cholesterol to fall.

(5) Variation of the inducing shock from 0·1 seconds to 1·0 causes no significant variation in the fall.

(6) Hypophysectomy lessens the effect of the convulsion, but the factors need further elucidation.

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