AUTONOMIC FUNCTION IN DEPRESSION: A MODIFIED METHACHOLINE TEST

By

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Review of the Literature

In 1948, Funkenstein, Greenblatt and Solomon reported on a test of autonomic function which is clearly a combination of tests used by earlier workers (McWilliam, 1925; Sachs, 1936; Myerson *et al.*, 1937; Gold, 1943; Altman, 1943). They reported a relationship between the changes in systolic blood pressure induced by injected adrenaline and methacholine and the clinical course, after electro-convulsive treatment in a group of psychiatric patients. This was followed by a considerable volume of work, published by Funkenstein and his colleagues, on the prognostic, diagnostic and other aspects of a test (the adrenaline-methacholine or Funkenstein test) which has aroused steadily increasing interest. The number of papers published has grown continually, and great variation exists in the techniques adopted and the scoring methods used. Some workers have confirmed the original findings whilst others have not. Thus the subsequent literature on the test has shown a pattern not unfamiliar in medicine; enthusiastic first publications followed by reports less satisfactory and more critical, and attempts to carry out a proper evaluation, producing many contradictory results.

Much of the literature has been well reviewed by Feinberg (1958), and his conclusions are that the predictive value of the test, either in the original or the abbreviated form (methacholine only), is inadequate for clinical purposes and that further work is necessary to define the parameters of the test. Those who accept the validity of the test consider that a marked hypotensive response to methacholine is associated with a good outcome after electro-convulsive treatment. Feinberg notes the relation between age and pronounced hypotensive response, and suggests that any relation between the test response and prognosis might merely reflect the change in distribution of mental disease which occurs with increasing age.

Further publications have appeared more recently. In scoring the results of the test, more objective criteria have been used as a basis for classification; the basal blood pressure, maximum fall in blood pressure, the "area of fall" to methacholine and the time for homeostasis are among the commoner variables used for this purpose. Probably the most satisfactory method of assessing the methacholine test is that reported by Hamilton (1959), which is based on the technique of fitting a straight line to the rising part only of the blood pressure curve. This method describes a given methacholine test in terms of 3 parameters: basal B.P., fall of B.P., and slope of line. In 1960 this author reported that the first two parameters were of good reliability, whilst the latter is less reliable.

Many have reported on the reliability of the test. Munro (1958) showed that the method of classifying curves is an important source of error, and should be replaced by some objective method of classification. Maas (1958), Sloane, Lewis and Slater (1957) and Blumberg (1960) reported favourably on the reliability of measurement, whilst Grosz and Miller (1958) found less

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favourable results. Good test-retest reliability (reliability of fluctuation) for the maximum drop in B.P. and area of fall was reported by Lotsof and Yobst (1957), Sloane, Lewis and Slater (1957) and Rickels and Ewing (1959); but a poor reliability, using Funkenstein's grouping, was indicated by the work of Brill, Richards and Berger (1958), Blumberg (1960) and others.

Other workers have been concerned with the prognostic validity of the test. Satterfield (1959) and Brill, Crumpton *et al.* (1959) failed to confirm Funkenstein's findings, whilst Hamilton and White (1960), scoring the response to methacholine in terms of basal B.P. and drop in B.P., found these measures to have no significant correlation with outcome after electro-convulsive treatment. Roberts (1959), however, found the maximum drop in B.P. to have the correlation of nearly 0·6 with treatment response; this is the highest value recorded and almost equals the reliability of the measure.

Little further work has been done on the use of the test as an index of clinical state. Davies (1960), using Gellhorn's classification, as well as measuring the basal B.P., maximum fall in B.P. and time for homeostasis, was unable to confirm Funkenstein's findings.

A few publications have dealt with the interpretation of the test. Curtis *et al.* (1960) investigated the excretion of adrenaline, noradrenaline and 17-hydroxycorticosteroids in a group of normal and psychiatric patients, and failed to find support for Funkenstein's theory. Sloane, Saffran and Cleghorn (1958) compared the eosinophil response and urinary excretion of 17-hydroxycorticosteroids after the injection of methacholine and corticotrophin, and found no evidence to support Gellhorn's theory of "central sympathetic reactivity". Rickels and Ewing (1959), however, reported favourably on Gellhorn's interpretation.

The influence of age on the Funkenstein test has been investigated by other workers. Lunde, Mansfield and Smith (1958), found a significant relation (at the 5 per cent. level) between improvement and age, in a group of 14 involutional psychotics. Roberts (1959) and Hamilton (1960), both reported a positive correlation between age and the two variables, basal B.P. and drop in B.P., but neither found any significant relation between age and prognosis after electro-convulsive treatment.

From this survey of the relevant literature on the Funkenstein test, the following conclusions may be drawn:

1. The reliability of the test varies according to the particular procedure adopted and the method of scoring used. With the modified form of the test, using methacholine only, and scoring the response in terms of certain parameters, the reliability is good. With the full Funkenstein test, using both adrenaline and methacholine (especially when both drugs are given the same day), and when the response is scored in terms of arbitrary classifications, the reliability is poor.

2. There is no evidence that the test, or any of its modifications, has any clinical value, i.e. has any value above clinical judgement. However, this does not mean it has no value. There is some evidence that the response of the blood pressure to methacholine is related to outcome after E.C.T. Although this prognostic value is low, examination of the data suggests that the test merits further investigation.

3. The interpretation and physiological implications of the test remain obscure. Funkenstein's adrenaline-noradrenaline hypothesis (Funkenstein *et al.*, 1952, 1954) lacks experimental confirmation, and although Gellhorn's

theory of central sympathetic reactivity (Gellhorn, 1953, 1955) has some support from animal studies, allied investigations on human subjects have not been done. It cannot be said that we have yet any adequate understanding of what the test measures.

4. It may be concluded that:

(*i*) If the test is ever to be of practical use, it will have to undergo considerable modification.

(*ii*) There is much scope for further investigations, not only into the prognostic and diagnostic relationships of the test, but also in order to clarify what the test actually measures.

The Present Investigation: Introduction

Although the clinical value of the Funkenstein test and its modifications has not been established, there are theoretical grounds for supposing that autonomic tests might be useful in depressive disorders. Thus it is widely believed that depressive illnesses bear some relation to temperament, and that the latter is possibly related to certain aspects of physiological function. Work by Campbell (1953), Hess (1954), Cleghorn (1955) and others has attempted to relate depressive states to disturbed function of the diencephalon and other areas of the brain associated with autonomic activity. Furthermore, characteristic disturbances of the autonomic nervous system frequently accompany depressive affect, and these are presumably manifestations of central processes having to do with those parts of the brain whose function is disturbed by the depressive process, whatever that may be. Thus it is reasonable to suppose that autonomic tests might be of some value. Such expectation, however, has not yet been realized.

Apart from the question of reliability, there are other possible reasons for this disparity between the practical and theoretical use of the Funkenstein test. Most investigations have used a heterogeneous group of patients, of different diagnoses, age and sex, and have not taken into account the effect of these variables. Methods of assessing the patients' condition and amount of improvement have been unsatisfactory. It is always possible that discrepant results arise because the test is valid in some (undefined) circumstances and not in others.

Another factor possibly responsible for such disparity is the lability of the autonomic nervous system, so that its general reactive functions, if any, tend to be obscured by its reaction to immediate circumstances. In assessing the response to autonomic tests, this lability constitutes a variable which must be considered, and it would appear advantageous to do such tests under conditions where it is minimized. Such a condition might prevail when autonomic activity is damped down by partial blockade of the autonomic ganglia.

Investigation of autonomic responses in the intact individual are difficult because autonomic mechanisms are complex and homeostasis is achieved by many different pathways. It is reasonable to assume that the effect of methacholine on the blood pressure initially represents the effect of this drug on peripheral end-organs, and is independent of its effect on higher centres. Under this assumption, some of the variables involved in the observed response are:

(*a*) end-organ sensitivity;

(*b*) receptor sensitivity (to the change in blood pressure);

(*c*) the state of the regulating mechanisms in the C.N.S.;

(*d*) the response of various organs to the output of the C.N.S.

While it may be possible to distinguish the contribution of these, and perhaps other variables, to the observed response, such studies have not yet been done. Therefore, it is possible that identical responses observed in different patients are the result of quite disparate mechanisms. The methacholine test would be better understood if it were investigated by the traditional method of interfering with the processes that occur during the test, in an endeavour to clarify what is going on. Interference with autonomic homeostatic processes would be achieved by partial blockade of the autonomic ganglia. Thus, aside from any practical value that might come from such investigations, there is the theoretical importance of investigating autonomic functions under different conditions and in various diseased states.

For the above reasons, it was decided:

1. To do the methacholine test under a condition of partial blockade of the autonomic ganglia.
2. To see how this affects the response to the test, and to examine the individual differences in relation to prognosis, diagnosis and interpretation of the test.
3. To do the test on a single diagnostic group of patients.
4. As far as possible, to use reliable, quantitative and objective methods for assessing relevant variables.

Method

Briefly, the investigation took the following form:

Fifty-four patients, of both sexes, each suffering from a primary depressive illness of sufficient severity to require electro-convulsive treatment, were carefully examined and assessed by means of an appropriate rating scale. Detailed clinical and social histories were obtained, and on aetiological grounds only, a diagnosis of Endogenous or Reactive Depression was made. The methacholine test was done, and on the next day was repeated after an intravenous injection of hexamethonium bromide. A course of electro-convulsive treatment was then given, and the patients were again assessed on the rating scale, 1 month and 3 months after the end of their course of treatment. The relationships between the results of the tests, the outcome after electro-convulsive treatment, and the diagnostic groups were then examined.

The basic considerations were:

When the methacholine test is given under a condition of partial blockade of the autonomic ganglia:

1. Are there any significant changes in the parameters of the test?
2. Is the prognostic value of the test increased?
3. Is there any relationship between the test and the diagnostic groups?
4. Finally, is any information gained towards clarification of what the test actually measures?

1. *Population*

Twenty men and 34 women were included in this study. The former and 28 of the latter were in-patients, the remaining 6 women being out-patients. Of the 54 patients initially investigated, 4 failed to complete their electro-convulsive treatment so that 50 were included in the final assessments after treatment. All were seen and treated in a psychiatric unit in a large general hospital, St. James's Hospital, Leeds.

An upper age limit of 65 years was made, in order to exclude older patients and thereby minimize the possibility of organic factors, due to ageing, being present. The mean age of the men was $53 \pm 6 \cdot 2$ Standard Deviation (with a range of 40–62 years), and that of the women was $46 \cdot 8 \pm 10 \cdot 0$ Standard Deviation (with a range of 20–63 years).

All were suffering from a primary depressive illness, considered to be of sufficient severity to warrant treatment by electro-convulsive therapy. A "depressive illness" was defined as a sustained primary mood disturbance, leading to subjective or objective inefficiency of mental activities, experienced in a mood of sadness, and usually with a diffuse, persistent lowering of interests and activity (Mayer-Gross, 1954). Electro-convulsive treatment was considered justified when symptoms had rendered the patient unfit for normal occupation, or constituted a serious social handicap, or had led to an apparently genuine attempt at suicide. Those patients treated in hospital had been admitted because of an attempted or seriously threatened suicide, or because they were so depressed that it was considered unsafe to treat them as out-patients.

Patients with depressive symptoms together with evidence of organic syndromes or other psychoses, were not included, nor were those who had had electro-convulsive treatment in the preceding six months.

2. *Clinical Examination and Diagnosis*

Each patient was seen personally, and if considered suitable for inclusion in the investigations, was then studied in more detail.

Routine physical examination was carried out on all patients, to exclude major physical disease. In view of the later use of methacholine and hexamethonium bromide, those suffering from asthma or heart disease were not included; essential hypertension, in the absence of cardiac, renal or cerebral involvement, was not considered a contraindication.

A detailed clinical and social history was obtained from each patient, supplemented by information from the relatives, and, where necessary, from a psychiatric social worker. The mental examination covered the usual points of appearance, behaviour, mood, talk and ideation, orientation, memory and insight etc.

On aetiological grounds only, the patients were classified into Endogenous, Doubtful Endogenous, Doubtful Reactive and Reactive Depressions. In Endogenous Depressions, no relevant precipitating factors could be elicited; where there might be some doubt about this, a diagnosis of Doubtful Endogenous Depression was made. In Reactive Depressions, relevant precipitating factors were clearly present; where there might be some doubt about the relevance of external stress, a diagnosis of Doubtful Reactive Depression was made. It might be considered that to make such a classification solely on aetiological grounds is unsatisfactory, since this could be done better if the symptoms were used as well. This classification was adopted because of its reasonably precise definition, and because the relation of detailed clinical

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features to the autonomic tests and outcome after electro-convulsive treatment, is intended to be the subject of a separate investigation.

3. *Assessment of Depression*

Each patient was assessed by means of a rating scale for depression (Hamilton, 1960), which, in effect, quantifies the results of the interview. This scale has a high reliability of measurement, is simple to use and has been found to be of particular value in assessing results of treatment. Practice in the use of the rating scale was first gained with depressed patients not included in this investigation.

The scale contains 17 symptoms which are measured either on 5 point or 3 point scales, the latter being used where quantification of the symptom is either difficult or impossible. The actual scores rated were then doubled and their total used in the calculations. The doubled score not only obviates any half-figures, but allows comparison with others using the scale and employing the technique of double assessment, in which the sum of assessments by two physicians is taken as the score for the patient (Hamilton and White, 1960).

Assessment of each patient was made on three occasions; before electro-convulsive treatment (Initial Score), usually during the first interview; 1 month (Final 1 Month Score) and 3 months (Final 3 Months Score) after the end of treatment.

4. *The Methacholine Test*

The Funkenstein test was used in its abbreviated form, with methacholine only. The technique adopted was based on that used by Sloane and Lewis (1956).

In the morning, and under fasting conditions, the systolic blood pressure was taken after the patient had been lying down for at least half an hour. Readings were taken for at least 5 minutes, at half minute intervals, until five consecutive readings within 8 mm. of Hg. were obtained; the average of these last five values was taken as the "Basal B.P.".

The patient was then given an intramuscular injection of 10 mg. methacholine chloride (in aqueous solution and used within 2 weeks of manufacture). The systolic blood pressure was then recorded at half minute intervals for 7 minutes, at 1 minute intervals for 6 minutes, and then at every 2 minutes to a total time of 25 minutes.

Atropine sulphate gr. 1/75 was kept at hand to check any apparently dangerous effects of the methacholine, and was used on three occasions: after the injection of methacholine one woman rapidly lost consciousness—this case was excluded entirely from the investigations; in 2 other women patients it was used to restore a low blood pressure which was still present at the end of the test.

The method adopted of assessing the results of this test was that described by Hamilton (1959). This is the method of curve-fitting, in which only the rate at which the B.P. returns to the basal level is considered. The rate of return of B.P. is adequately dealt with by fitting a straight line to the rising part of the curve, and this straight line is used as a substitute for the empirical curve. Thus, using the data of a test to derive a straight line which best fits the curve of rising B.P., any given methacholine test is described in terms of three parameters—Basal B.P., Drop in B.P. (as measured by the point where the straight line cuts the ordinate at 2 minutes after the injection), and the Slope of the

Line. It was decided to use also the time of return of the B.P. to the basal level, i.e. the Time for Homeostasis (as measured by the point were the straight line cuts the horizontal axis). This variable is dependent on both the Drop in B.P. and the Slope of the Line, and for this reason was thought to be a useful measure. These determinations were carried out as follows:

The information about each patient was first put into graph form, on 1 mm. squared paper, systolic B.P. in mm. Hg. on the ordinate, and time in minutes on the abscissa. The Basal B.P. was also indicated, so that the variations in blood pressure and return to resting level were clearly visible. Each curve was then carefully examined and a decision was made about how many measurements to include to cover the rising part of the curve, and this part only—this is not a major problem, because a few measurements more or less will alter the slope of the fitted line by only a very slight amount. The straight line was then calculated by the method of least squares. It is represented by the formula $Y=aX+b$, where Y is the blood pressure at time X, and b is the point where the line cuts the blood pressure (vertical) axis. The figure b is a hypothetical drop of blood pressure, since it is supposed to be present at the time of injection; instead, the drop of blood pressure is taken as a point on the line, 2 minutes after the injection, and the pressures recorded at $\frac{1}{2}$, 1 and $1\frac{1}{2}$ minutes after the injection are not used for calculating the line. The a, which measures the slope, represents the tangent of the angle between the line and the horizontal axis.

Fortunately facilities were given to use the electronic computing machine at the Leeds University for these calculations. After being given the relevant data and instructions, the machine supplied the three measures, blood pressure at 2 minutes after injection, the time for homeostasis and the slope of the line. The latter, given as a tangent, would give a skewed distribution of slopes, and was therefore converted to degrees (from a table of tangents). The drop in blood pressure was then found by simple deduction of the 2 minute level from the basal level. Finally, each straight line was drawn on its own curve on the graph paper, and examined for correctness.

5. *The Hexamethonium-Methacholine Test*

In this test, an intravenous injection of hexamethonium bromide was first given, and when the blood pressure had reached a stable level, 10 mg. methacholine chloride was injected intramuscularly and the blood pressure recorded as for the methacholine test.

Pilot Study No. 1

This was done to determine the amount of hexamethonium bromide which was both safe and adequate. A sufficient amount was required to produce evidence of a definite effect on the blood pressure, as shown by a drop in basal blood pressure (due to hexamethonium alone) as well as by an apparent change in the response to methacholine; such effects were regarded as evidence of partial blockade of the autonomic ganglia. On the other hand, since both hexamethonium and methacholine are potent hypotensive agents, their combined administration could be dangerous, and therefore it was considered wise to use the smallest adequate dose of the former.

The subjects of this study were 12 patients, of both sexes, suffering from primary depressive illnesses, for which electro-convulsive treatment was considered necessary. Starting with 5 mg. of each drug, and then slowly increasing the dose, each patient was first given the methacholine test; on the next day, after establishing the basal B.P. level, hexamethonium was injected intravenously and the blood pressure recorded at ½ minute intervals, for at least 5 minutes, until five consecutive readings within 8 mm. of Hg. were obtained. Methacholine intramuscularly was then injected, and the blood pressure recorded at intervals for 25 minutes. The doses were increased as follows:

No. of Patients	Methacholine Test mg.	Hexamethonium-Methacholine Test mg.	mg.
2	5	5	5
2	10	5	10
2	10	10	10
6	10	15	10

After examination and comparison of the blood-pressure curves of both tests, it was decided that 15 mg. of hexamethonium bromide was an adequate dose. The last 6 patients of this pilot study were included in the chief investigation.

Pilot Study No. 2

For the proper evaluation and interpretation of the hexamethonium-methacholine test, it is essential that the ganglion-blocking action of the former drug persists for the duration of the methacholine test which follows it. Whilst the work of Harrington (1953) indicates that this is in fact true, it was considered wise to confirm this point.

Eight in-patients, 5 women and 3 men, and each suffering from a primary depressive illness, formed this study; 6 of them subsequently received electro-convulsive treatment. Under resting and fasting conditions, a basal B.P. level was first established. Hexamethonium 15 mgm. intravenously was then given to each patient, and the systolic blood pressure recorded at ½, 1 and 2 minute intervals, for periods varying from 30 to 75 minutes; the results were plotted on graph paper and the curves carefully examined. In no case did the blood pressure rise to basal level during the observation period, and the curves showed a fairly stable blood pressure level after an initial period of 5 to 10 minutes.

These results provided adequate confirmation that the duration of action of hexamethonium bromide was suitable for the purpose required, especially since in many cases the rising part of the B.P. curve (after methacholine) did not extend to the full 25 minute observation period. This study also indicated that the new basal level (after hexamethonium) is usually established within 5 to 10 minutes of the injection.

The procedure finally adopted for the hexamethonium-methacholine test was as follows:

In the morning, and under fasting conditions, the systolic blood pressure was taken after the patient had been lying down for at least half an hour. Readings were taken for at least 5 minutes, at ½ minute intervals, until five consecutive readings within 8 mm. of Hg. were obtained; the average of these last five readings was taken as the basal level (before hexamethonium). Hexamethonium bromide 15 mg. intravenously was then given over a period of ½ to 1 minute, and the systolic blood pressure was recorded at ½ minute intervals, for at least 5 minutes, until five consecutive readings within 8 mm. of Hg. were obtained; the average of these last five readings was taken as the

"Basal B.P." for this test. Methacholine 10 mg. intramuscularly was then given and the systolic blood pressure recorded at intervals for 25 minutes, as for the methacholine test.

Methedrine 30 mgm. and atropine sulphate gr. 1/75 were kept at hand to counteract any dangerous hypotensive effects. After injection of hexamethonium, a male patient, moderately hypertensive, showed a marked fall in blood pressure, and needed treatment with methedrine; the next day, the hexamethonium was given more slowly and the full test was completed without any untoward effect. Another patient was given atropine sulphate to relieve persistent hypotension at the end of the test. Apart from these two instances, no major difficulties were encountered using this technique, although the very marked hypotensive effect of hexamethonium and methacholine together was noted in several patients.

The results were plotted on graph paper, the curves examined, and the regression lines for the rising part of each curve calculated, as for the methacholine test (see Figure 1). The Basal B.P., the Drop in B.P. (difference between B.P. before and 2 minutes after the methacholine injection), the Slope and the Time for Homeostasis were noted for each patient.

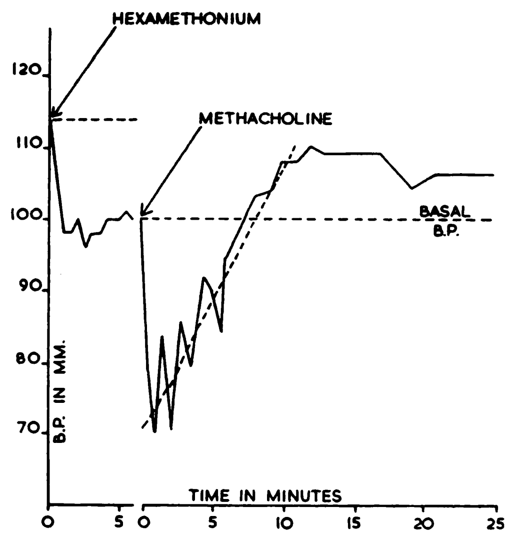

FIG. 1.

It was initially intended to give the methacholine test first in half the patients, with the order of the tests reversed in the other half. However, in view of the severe hypotension observed when the two drugs were given together, it was later decided to give the methacholine test first to each patient, so that some indication of any dangerous hypotension would be given. Of the 54 patients, 38 were given the methacholine test first, and 16 the hexamethonium-methacholine test first, with an interval of 1 or 2 days between the two tests.

6. Electro-convulsive Treatment and Reassessments

No formal psychotherapy was given, other than the usual pleasant doctor-patient relationship, together with ordinary discussion of personal difficulties, reassurance and explanation. Hypnotics at night for sleep (usually sod. amytal gr. 3–6) were the only drugs allowed.

Electro-convulsive treatment was in all cases given by the " Ectron " apparatus, using an alternating current, 115 volts, for 1–2 seconds. No form of cerebral stimulation was employed. Atropine sulphate gr. 1/75 intramuscularly was given 30 to 45 minutes before treatment, which was modified by thiopentone 0·2–0·5 G. intravenously, followed by suxamethonium chloride, 15–40 mg., given through the same needle. Insufflation with oxygen, by means of a hand positive-apparatus, was available to prevent hypoxia until normal spontaneous respiration occurred. Electro-convulsive treatment was given twice weekly, and continued until it was considered that the maximum benefit had been obtained. There was no "course" or fixed number of treatments. Treatments given varied from 6 to 12, with an average value of 6–7.

A careful note was made of the number of treatments given and the date of the last one. Most in-patients were discharged from hospital within 3–4 weeks of the last treatment. Reassessments, using the rating scale, were then carried out on every patient, 1 month after the end of treatment, and again 2 months later, i.e. 3 months after the end of treatment. These were done at the "follow-up" clinic, apart from a few cases where the patient was seen at home, having failed to appear at the clinic. During this 3 months reassessment period, care was taken that no drug, other than hypnotics for sleep, was given.

Of the 54 patients, four failed to complete their electro-convulsive treatment, so that 50 were included in the final assessments after treatment. Treatment was stopped in one case, with moderate hypertension, on account of increasing difficulty in recovery after treatment, and in another case because of the production of marked depersonalization symptoms. Of the two remaining patients, one refused further treatment and the other left hospital, against advice, before her treatment was ended. All these four patients were women and three were diagnosed as Reactive Depressions.

Results

1. *Parameters of the Two Tests Compared*

The effect of hexamethonium on the 4 parameters of the methacholine test was examined. In each case the change produced by hexamethonium was recorded, by subtracting the methacholine value from the hexamethonium-methacholine value. The null hypothesis states that the means of these changes do not differ significantly from zero, and Student's t test was used to evaluate this (Table I).

Table I

Significance of a Single Mean

	Male (n = 20)		Female (n = 34)	
	t	P	t	P
Basal B.P.	4·24	< ·001	6·75	< ·001
Drop in B.P.	< 1	Not Signif.	< 1	Not Signif.
Slope	< 1	,, ,,	< 1	,, ,,
Time for Homeostasis ..	< 1	,, ,,	< 1	,, ,,

For both men and women, the change in Basal B.P. with hexamethonium is highly significant, as would be expected; the means, without and with hexamethonium, are in the former, 131·6 and 115·5, and in the latter, 133·5 and 113·5 respectively. The other parameters show no significant changes in their means.

Two sets of values, with equal means, may have different spreads or variabilities. So, next, the variances of the parameters (excluding Basal B.P.), were compared. Since the two sets of measures are from the same individuals, the usual test of significance of the difference between the variances is not applicable, as this presupposes that the variables measured are independent. Another test was used (Biometrika, 1939, 30, pp. 13–19), based upon the fact that given any two variables X and Y, the correlation between the derived variables (X+Y) and (X−Y) will be zero if the variances of X and Y are equal. Thus a test of significance of the correlation between the derived variables is equivalent to a test of significance of the difference between the variances. Table II shows the correlations between (X+Y) and (X−Y), for the various measures, where X=hexamethonium-methacholine test measure and Y= methacholine test measure.

TABLE II

Correlation Co-efficients for $(X+Y)$ and $(X-Y)$

	Male (n=20)		Female (n=34)	
Drop in B.P.	·156	Not Signif.	·188	Not Signif.
Slope	−·159	,, ,,	−·035	,, ,,
Time for Homeostasis	−·284	,, ,,	·021	,, ,,

(n=19, since case 40 was excluded because of high value of 219)

All tests show no significant difference from zero, even at the 10 per cent. level. Therefore the variances of the parameters of the two tests do not differ significantly.

Thus, considering the groups as a whole (Tables I and II), no significant changes occur in the parameters of the methacholine test as a result of partial blockade of the autonomic ganglia by hexamethonium (excluding Basal B.P.).

TABLE III

Comparison of Means

Methacholine Test	Males (n=20) Increased by Hexamethonium	Decreased by Hexamethonium	t	P
Drop in B.P.				
No. in Groups ..	10	10		
Group Means ..	21·6	27·6	1·17	Not Sig.
Slope				
No. in Groups ..	9	11		
Group Means ..	25·6	58·4	5·14	< ·001
Time for Homeostasis				
No. in Groups ..	10	8		
Group Means ..	7·6	19·6	3·18	< ·01

(n=18, since 1 case was not changed by Hexamethonium and 1 case (No. 40) was excluded because of an unusually high value of 219 minutes)

TABLE IV

Comparison of Means

Methacholine Test	Females (n=34) Increased by Hexamethonium	Decreased by Hexamethonium	t	P
Drop in B.P.				
No. in Groups ..	16	18		
Group Means ..	19·9	35·2	2·65	< ·02
Slope				
No. in Groups ..	15	19		
Group Means ..	26·4	51·3	5·51	< ·001
Time for Homeostasis				
No. in Groups ..	16	16		
Group Means ..	12·6	26·6	4·14	< ·001

(n=32, since 2 cases were not changed by Hexamethonium)

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The parameters of the methacholine test were then examined for the *kind* of change brought about by hexamethonium. Each group, male and female, was classified into two sub-groups, depending on whether the particular parameter was increased or decreased by hexamethonium, and these sub-groups were then compared (Table II and Table IV).

The difference for the Drop in B.P. (male) is not significant, but all the remainder are significant at better then the 2 per cent. level. It is seen that the sub-groups increased by hexamethonium all have lower means than those decreased by this drug. Hexamethonium then, appears to have a dual and opposite effect on the parameters of the methacholine test, depending on the initial level of these measures. This effect is less consistent in the case of the Drop in B.P.—in the male group it fails to reach a significant value, whilst in the females the level of significance is lower than those for the other parameters.

Thus, whilst the groups as a whole show no significant changes in the methacholine test when the autonomic ganglia are partially blocked, further examination reveals that the changes which do occur are not random ones, and in an individual are related to the initial level of the particular parameter. This is indicated by the presence of sub-groups, which differ in the direction of the change brought about by hexamethonium.

2. *Relation of the B.P. Measures to Prognosis* (*After E.C.T.*)

The correlation co-efficients for the Final Scores (1 month and 3 month) and the parameters of the two tests were calculated (Table V).

The results are disappointing. Out of 48 correlations none are significant at the 5 per cent. level. Only 3 attain any significance, and then barely so. Furthermore, 2 of them relate to the Basal B.P. which, strictly speaking, is not a measure of the methacholine test itself. These few correlations, all of low significance, might well have occurred by chance.

TABLE V

Correlation Co-efficients for Test Measures and Outcome

Methacholine Test	Basal B.P.	Drop in B.P.	Slope	Time for Homeostasis
Final 1 Month Score				
Male (n=20)	·037	·177	·038	−·162
Female (n=30)	·123	·179	−·103	·310†
Total..	·081	·183	−·053	−·008
Final 3 Month Score				
Male (n=19)	·164	·088	·074	−·122
Female (n=30)	−·124	−·032	·050	·036
Total..	−·050	·006	·017	−·056
Hexa.-Meth. Test				
Final 1 Month Score				
Male (n=20)	·132	−·022	·113	−·193
Female (n=30)	·309†	·256	−·103	·177
Total..	·254*	·173	·017	·106
Final 3 Month Score				
Male (n=19)	−·038	−·201	·088	−·264
Female (n=30)	−·046	·005	·080	−·133
Total..	−·051	−·026	·066	−·122

† P=·1   * P=<·1

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These results clearly indicate that, in the group of patients studied:

(*a*) The methacholine test has an insignificant prognostic value.

(*b*) Under a condition of partial blockade of the autonomic ganglia, the prognostic value of this test is not increased.

It is perhaps interesting to note that of the only 3 significant correlations, 2 are derived from giving hexamethonium alone—such a procedure is far simpler than the methacholine test and might well deserve further examination.

Further correlation co-efficients, for the Final Scores and other measures derived from the two tests, were then determined (Table VI).

TABLE VI

Correlation Co-efficients for Test Measures and Outcome

	Change in Drop	Change in Slope	Change in Time for Homeos.	Drop with Hexa. only	Total Drop (Hexa.+ Meth.)
Final 1 Month Score					
Male (n=20)	−·174	·053	·134	·002	−·016
Female (n=30) ..	·058	·015	−·095	−·251	−·048
Total	−·015	·032	·041	−·266*	−·033
Final 3 Month Score					
Male (n=19)	−·264	·003	·088	·244	−·005
Female (n=30) ..	·042	·012	−·129	−·186	−·156
Total	−·033	·026	·014	−·097	−·095

* P=<·1

Out of 30 correlation co-efficients, none are significant at the 5 per cent. level. Only 1, for the Drop with Hexamethonium and Final 1 Month Score (Total), is significant at all (P=<·1). Whilst this might well be due to chance, it is perhaps worth noting that again, this measure is derived from giving hexamethonium alone.

3. *Relation of the B.P. Measures to the Diagnostic Groups*

The difference in the various B.P. measures among the four diagnostic groups, were then determined, by the method of analysis of variance (Table VII and Table VIII).

TABLE VII

Comparison of the Diagnostic Groups—Analysis of Variance

	Males	
	F Ratio	P
Methacholine Test		
Basal B.P.	<1	Not Significant
Drop in B.P.	<1	,, ,,
Slope	<1	,, ,,
Time for Homeostasis	<1	,, ,,
Hexameth.-Methachol. Test		
Basal B.P.	1·4	,, ,,
Drop in B.P.	<1	,, ,,
Slope	<1	,, ,,
Time for Homeostasis	<1	,, ,,
Other B.P. Measures		
Change in Drop	<1	,, ,,
Change in Slope	1·48	,, ,,
Change in Time for Homeostasis	1·11	,, ,,
Drop with Hexameth. only	<1	,, ,,

TABLE VIII

Comparison of the Diagnostic Groups—Analysis of Variance

	Females	
	F Ratio	P
Methacholine Test		
Basal B.P.	<1	Not Significant
Drop in B.P.	2·03	<·2
Slope	2·86	·05
Time of Homeostasis	2·26	<·2
Hexameth.-Methachol. Test		
Basal B.P.	1·14	Not Significant
Drop in B.P.	1·58	" "
Slope	<1	" "
Time for Homeostasis	1·86	<·2
Other B.P. Measures		
Change in Drop	<1	Not Significant
Change in Slope	2·08	<·2
Change in Time for Homeostasis	3·57	<·05
Drop with Hexameth. only	<1	Not Significant

None of the measures indicate any differences between the male diagnostic groups. In the females, two measures differ significantly between the four groups; Slope (Methacholine test), P=·05, and Change in Time for Homeostasis, P=<·05. Details for these are shown in Table IX.

TABLE IX

Comparison of the Diagnostic Groups—Analysis of Variance

Females

	Endo-genous	Doubtful Endo-genous	Doubtful Reactive	Reactive	F Ratio	P
Methacholine Test						
Slope						
No. in Groups	9	5	9	11		
Group Means	40·5	22·4	49·5	40·7	2·86	·05
Change in Time for Homeostasis						
No. in Groups	9	5	9	11		
Group Means	9·5	−10·2	5·5	−8·1	3·57	<·05

Although the Slopes differ significantly, this is chiefly due to the low mean of the Doubtful Endogenous group; the mean of the Endogenous group (40·5) is identical to that of the Reactive group (40·7). With regard to Change in Time for Homeostasis, whilst the Endogenous group has a high mean (9·5) and the Reactive group has a low mean (−8·1), the Doubtful Endogenous (−10·2) and the Doubtful Reactive (5·5) groups are the reverse of what might be expected. Thus, despite the apparent significant differences, no clear picture emerges.

Whilst there is no strong evidence of any relation between the B.P. measures and the diagnostic groups, it is seen that in the females (Table VIII), 6 of the 12 variance ratios are significant at better than the 20 per cent. level, but none in the males (Table VII). These results might possibly indicate some difference between the male and female diagnostic groups.

Discussion

Changes in the Methacholine Test with Hexamethonium

The lack of any significant changes in the parameters of the methacholine test when this is done after hexamethonium (Tables I and II), means that these measures are not affected by partial blockade of the autonomic ganglia, and are therefore not dependent on the activity of the autonomic nervous system (as indicated by activity in the autonomic ganglia). Whilst this conclusion applies to each group (male and female) as a whole, it is true only if there are no sub-groups affected in opposite directions by hexamethonium. The sub-groups based upon aetiological factors (diagnostic groups) show no such differentiation. There is, however, evidence that such sub-grouping may be possible, because if the groups are classified according to whether the test parameters are increased or decreased after hexamethonium, significant differences are found between some of these sub-groups (Tables III and IV). There is thus some evidence that the test parameters are changed by hexamethonium and therefore affected by autonomic activity; consequently the present conclusion must be qualified in this respect.

The evidence for such a change in the Drop in B.P. is less than that for the other parameters—in the men (Table III) there is no significant difference between the sub-groups, and in the women (Table IV) the difference is at a lower level of significance ($P = < \cdot 02$) than that for the Slope ($P = < \cdot 001$) and Time for Homeostasis ($P = < \cdot 001$).

Injected methacholine exerts its effects by acting on the peripheral end-organs (arterioles), and it is a reasonable hypothesis that the initial response to this drug i.e. the Drop in B.P., occurs as a result of this peripheral effect, and is independent of higher central (autonomic) activity. The absence of a significant change in this parameter, when methacholine is given under a condition of partial blockade of the autonomic ganglia, supports this hypothesis. Further evidence in support of this reasoning is given by Goodman and Gillman (1955). They report that in hypertensive patients whose blood pressure falls to normal during a control rest period, methacholine, given after the rest period, causes a greater fall in blood pressure than occurs in normotensives. Apparently the peripheral vessels in patients with hypertension respond more to the drug than do those of normal persons, and the absolute level of the blood pressure is not the important factor conditioning the fall in blood pressure after methacholine. Thus the peripheral effect of methacholine is emphasized.

The Drop in B.P. after methacholine, then, appears to be unrelated to central autonomic activity, particularly in the male group, and is probably chiefly related to peripheral end-organ sensitivity. Since this measure is the only reliable component of the methacholine test, (the Basal B.P., although reliable, is not a true component of this test), and since there are no grounds for believing that peripheral end-organ sensitivity is related to the outcome after E.C.T., the theoretical foundations of the methacholine test are strongly suspect. On this basis, no prognostic value of this test would be predicted, at any rate for the male group.

The lack of any significant change in the Slope and Time for Homeostasis after hexamethonium is perhaps more surprising, since homeostatic mechanisms, and therefore autonomic activity, must be involved in the restoration of the blood pressure to basal level. These parameters are not of good reliability (Hamilton, 1959), and this could account for such results. Moreover, the Slope and Time for Homeostasis differ significantly among the sub-groups based on the direction of change brought about by hexamethonium (Tables III and IV); this provides evidence that these measures are changed by partial blockade of the autonomic ganglia and are therefore related to autonomic activity.

With hexamethonium, 24 of the 54 patients show an increase in Slope and 24 a decrease in Time for Homeostasis (Tables III and IV), whilst 18 show both these changes. This means that, in these patients, homeostasis is more rapid when the autonomic ganglia are partially blocked. Such a finding cannot easily be explained by Gellhorn's theory. According to Gellhorn (1953), central sympathetic functions are actively concerned in the restoration of the blood pressure after methacholine, whilst parasympathetic activity plays no significant role; thus homeostasis should be more rapid in no single case, since hexamethonium can only reduce activity in the sympathetic ganglia.

How then, are these findings explained? If it is postulated that in these patients homeostasis is disordered, so that the homeostatic response to the drop in B.P. after methacholine is one of *diminished* sympathetic activity, then partial blockade of the autonomic ganglia would be expected to produce a more rapid homeostatic effect. Thus it is true that a consideration of sympathetic activity alone could, theoretically, account for such findings.

However, hexamethonium affects both the sympathetic and parasympathetic ganglia, and both components of the autonomic nervous system are likely to be involved in homeostasis. On this basis, if the autonomic ganglia are partially blocked in a patient with a relatively high level of para-sympathetic activity, as compared with sympathetic activity, then the effect could be one of sympathetic preponderance. In such a case, a more rapid restoration of the blood pressure after methacholine would occur, chiefly by means of an increase in heart rate via relaxation of vagal inhibition. Alternatively, in patients with a relatively high level of sympathetic activity or with a normal autonomic balance, partial blockade of the autonomic ganglia would result in a delayed homeostatic effect, due to a lowering of sympathetic activity. In this way, then, sub-grouping according to the *direction* of change produced by hexamethonium becomes understandable. Patients in those sub-groups showing delayed homeostasis after hexamethonium (i.e. diminished slope and increased time for homeostasis) would be reasoned to have a relative sympathetic over-activity, whereas those with a more rapid homeostasis after hexamethonium (i.e. increased slope and diminished time for homeostasis) would be characterized by a relative parasympathetic preponderance.

If this reasoning is correct, it follows that both the sympathetic and parasympathetic nervous systems are actively concerned with the return of the blood pressure to basal level in the methacholine test. In any case, the data are not consistent with Gellhorn's theory of central sympathetic reactivity; this serves to illustrate that conclusions obtained from experiments on anaesthetized animals do not necessarily apply to conscious human subjects.

Examination of the relation between these sub-groups and prognosis and other clinical factors would be of much interest, and might provide further support for the existence of such sub-grouping. This is intended to form part of a later investigation, concerned with the detailed clinical features and the blood pressure measures.

(Some preliminary investigations of this nature have been completed for the sub-groups based on the direction of change of the slope after hexamethonium. With regard to prognosis, no difference in outcome after E.C.T. was found between these groups, for both males and females. Considering the clinical features (symptoms on the rating scale), however, comparison of the two sub-groups in the females for the retardation score showed a difference significant at better than the 1 per cent. level ($t=3\cdot06$; $P=<\cdot01$). The mean retardation score for the sub-groups with increased slope after hexamethonium was 3·2, whilst that for the sub-group with decreased slope after hexamethonium was 0·89. The correlation between retardation score and change in slope after hexamethonium was 0·49, which is significant at better than the 1 per cent. level. Thus a more rapid homeostasis after partial blockade of the autonomic ganglia appears to be related to the presence of retardation in the females. On the basis of previous reasoning, this indicates an association between retardation and parasympathetic preponderance. This finding is in the direction one might have predicted.)

Prognosis

In view of the conclusions already reached, the lack of prognostic value of both the methacholine and hexamethonium-methacholine tests is not surprising. It is perhaps interesting to note, however, that of the only three significant correlations (Table V), one is for Time for Homeostasis and two are concerned with the Basal B.P. Both these measures are more likely to be related to autonomic activity, and therefore perhaps to prognosis, than the Drop in B.P.

The Diagnostic Groups

Although the tests have no prognostic value, there is evidence that they may have some relation to the diagnostic groups in the females (Tables VIII and IX). Of the 6 significant variance ratios, only 2 are at the 5 per cent. level of significance. These are the methacholine test Slope ($P = \cdot 05$), and the Change in Time for Homeostasis ($P = < \cdot 05$). Assuming these results are significant and not due to chance, and with the reservations already mentioned (page 637), it is possible that the methacholine test, with and without hexamethonium, might prove to have value as an index of certain clinical factors. In this respect, examination of the relation of the B.P. measures to the detailed clinical features seems to be indicated.

Summary

1. The relevant literature on the Funkenstein test is reviewed, and conclusions are reached about the current status of this test.

2. The rationale of giving the methacholine test under a condition of partial blockade of the autonomic ganglia is discussed, and the method is described of doing this modified form of the test, together with the standard test, on a group of depressed patients, as well as assessing them before and after electro-convulsive treatment.

3. An examination is made of the relation between the test measures, the outcome after E.C.T., and the diagnostic groups. The results show no difference between the measures of the two tests considering the groups as a whole, no prognostic value of these measures, but a possible relation to the diagnostic groups in the females.

4. The implications of these results are discussed, especially in relation to the theoretical basis of the Funkenstein (methacholine) test. It is argued that the only reliable component of this test—the drop in blood pressure—is chiefly related to peripheral end-organ sensitivity and is therefore unlikely to predict the response to E.C.T. Evidence is also given for doubting the validity of Gellhorn's theory that the methacholine test is an index of central sympathetic reactivity only.

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