Summary

A case of cantharidin poisoning in which death occurred is described and clinical aspects of this condition are briefly discussed.

Our thanks are due to Professor M. L. Rosenheim for permission to publish this case, and to Professor C. Rimington and Dr. Donald Teare for their helpful advice.

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CALCIUM CHLORIDE AND ADRENALINE AS BRONCHIAL DILATORS COMPARED BY SEQUENTIAL ANALYSIS

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It has been claimed by Howell (1953) that the inhalation of calcium chloride in the form of an aerosol is useful for relieving the bronchial spasm often present in patients with acute or chronic bronchitis. The use of this substance was suggested by Charlier and Philippot (1949), who noted bronchodilatation in healthy men when a 20% solution of calcium chloride was given by inhalation, the measurement of the bronchodilatation being made by means of tidal-air tracings.

The wheezing associated with acute bronchitis usually subsides rapidly in hospital as a result of treatment with appropriate chemotherapy, and it is probable that the even temperature of a hospital ward has, in itself, a therapeutic action. Wheezing in acute attacks of asthma generally responds to adrenaline by injection or inhalation, but wheezing associated with chronic bronchitis (Oswald, Harold, and Martin, 1953) and other chronic pulmonary diseases, such as coal-workers' pneumoconiosis, is frequently more difficult to relieve, although adrenaline is often effective. It is for this latter type of bronchial spasm that an alternative bronchodilator agent would be useful, and, as a solution of calcium chloride is cheap and easy to prepare, it was decided to carry out a controlled trial in order to assess its bronchodilator effect.

Method of Trial

The simplest and most satisfactory form of trial would have been one in which each subject's wheeze was assessed before and after an inhalation of calcium chloride. However, it would then have been impossible to distinguish between the real effect of calcium chloride and the psychological effect of any form of therapy unless a dummy inhalation were introduced. Ethical considerations precluded any such procedure; subjects suitable for the trial had a specific disability which, it was known, could almost always be relieved by established methods. Accordingly, it was decided that the trial should be a comparative one between the effects of calcium chloride and a known bronchodilator drug, adrenaline, on the same subjects. Adrenaline was used as the standard for comparison since it is generally accepted as being both effective and capable of being used over prolonged periods of time. Its only disadvantages are its occasional undesirable effects on the circulatory system and its lack of stability. It is the drug which would be deposed from first place in the treatment of bronchial spasm by the discovery of a more effective bronchodilator agent.

Suitable subjects with chronic bronchial spasm of diverse aetiology were drawn from the ward of the Pneumoconiosis Research Unit and the general medical wards of Llandough Hospital. Each subject had been in hospital for at least a week, all had chronic bronchial spasm clinically detectable by stethoscopy, none was having chemotherapy for a respiratory infection, and all other bronchodilator drugs were stopped 12 hours before the assessments were made. The assessment of the drugs was made in terms of the expiratory flow rate (E.F.R.)—the timed vital capacity of Gaensler (1951)—which is a measure of the maximum rate at which a subject can expel air from his lungs during a forced expiration. Although this test takes only a few seconds, the flow rate is expressed in litres per minute, normal values in young men lying between 150 and 200 litres per minute. All of our subjects, however, had values of less than 50 litres per minute, these low figures being caused partly by bronchial spasm and partly by chronic pulmonary disease producing a deficient "bellows action" of the lungs. The E.F.R. is known to be extremely sensitive to the presence of bronchial spasm, and may be used as a measure of it (Kennedy and Stock, 1952; Kennedy, 1953) because variations in the bronchial lumina are accompanied by corresponding variations in ventilatory ability. The test can be used repeatedly without causing the fatigue associated with the more conventional forms of maximum voluntary ventilation tests.

The E.F.R. of each subject was determined in the morning as the mean of three successive readings. A 15-minute inhalation of 3 ml. of either adrenaline or calcium chloride was given from a Collison inhaler at a flow rate of 10 litres of oxygen per minute. Neither the patient nor the observer knew which substance was given, the decision depending on the toss of a coin. The E.F.R. was again determined on the completion of the inhalation, and on the evening of the same day the procedure was repeated, using the other substance. The subject was questioned and a clinical assessment of the degree of bronchial spasm was made before and after each inhalation. The errors inherent in making such assessments as opposed to purely objective ones are discussed below.

The trial was carried out on a sequential basis (Wald, 1947), the results being assessed as soon as each subject was tested, the subjects being taken as they became available (effectively at random), and the decision made to continue the trial or conclude it in accordance with a predetermined plan. This plan was based on the following rules, arrived at after careful discussion with our colleagues of the theoretical and practical qualities of calcium chloride and adrenaline:

1. If calcium chloride caused subjects to gain, on the average, 10 litres per minute of E.F.R. more than they gained on adrenaline, calcium chloride would be regarded as superior to a material degree.

2. If the gain of E.F.R. was no greater with calcium chloride than with adrenaline, adrenaline would be preferred in view of its well-established virtues.

3. The risk of making either of the decisions (1 or 2) wrongly as a result of chance fluctuations in the experimental results should be only 1%.

From this plan the interpretation of the sequence of results obtained from successive subjects can be made

completely unambiguous. Further, it can be shown that the average number of subjects required to reach decisions 1 or 2—that is, to establish the one conclusion or the other with statistical significance—is less than in the usual non-sequential trial. This is an important consideration, particularly so in the present investigation, in which suitable subjects were expected to become available for inclusion only infrequently.

One modification of the rules of the trial had to be adopted. The orthodox sequential trial requires a knowledge of the variability of observations on the same subject in the same circumstances, expressed as a standard error. This knowledge was lacking, and only a rough estimate, that the standard error of the E.F.R. was 10 litres per

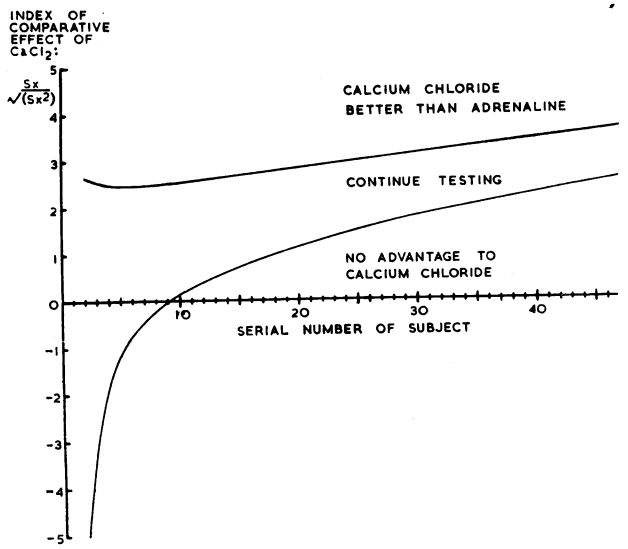

FIG. 1.—Boundaries for the sequential trial.

minute, was available. Accordingly rule 1 was re-phrased, and the material difference which should cause calcium chloride to be accepted as preferable was called one standard error unit, it being appreciated that the trial results might reveal that true standard error to be greater or less than 10 litres per minute. The trial was then carried out by means of the sequential t-test described by Rushton (1950). The difference between the gain of E.F.R. resulting from calcium chloride inhalation and that resulting from adrenaline inhalation was noted as each subject's results became available and the cumulative total worked out. In addition the cumulative total of the squares of these differences was obtained. The value of the ratio of the first of these totals to the square root of the second—that is, $Sx/\sqrt{(Sx^2)}$—was plotted (Fig. 1). So long as the successive points lay between the two curved boundaries the trial was continued; as soon as one fell outside, the trial was concluded by making one of the decisions contained in rules 1 and 2. The crossing of the upper boundary would indicate that calcium chloride satisfied the requirements of rule 1; the crossing of the lower boundary that it was no better than adrenaline (note that no provision was made in the trial for testing the hypothesis that calcium chloride was significantly *worse* than adrenaline).

The boundaries were so calculated that the risks of error specified in rule 3 are satisfied by this procedure. They were derived from the approximation given by Rushton (1950) for the logarithm of the likelihood ratio (case when $\delta=0$, $\delta'=1$):

$$\log l = \tfrac{1}{4}u^2 - \tfrac{1}{2}n + u\sqrt{n(1-1/4n+u^2/24n)}$$
where $u = Sx/\sqrt{(Sx^2)}$

The index $Sx/\sqrt{(Sx^2)}$ can be interpreted as a measure of the comparative effect of calcium chloride; it will be large and positive when the effect is consistently good, large and negative when it is consistently bad.

The trial was begun using calcium chloride in a concentration of 20% and adrenaline in a concentration of 1/200, both drugs being administered from a Collison inhaler at a flow rate of 10 litres of oxygen per minute. The details of the subjects and the results are given in Table 1, and the test criterion is plotted on Fig. 2, which is a part of Fig. 1 shown on a larger scale. It will be seen that in no single case did calcium chloride cause an increase of E.F.R., while each case showed the expected increase after adrenaline. The lower boundary in Fig. 2 was crossed

TABLE I.—*Result of First Trial with 20% Calcium Chloride*

(1) Subject	1	2	3	4
(2) Sex	M	M	F	F
(3) Age (years)	33	58	44	43
(4) Condition	P.F.U.O.	A., C.B.	A., C.B.	A., C.B.
(5) E.F.R. on calcium chloride (l./min.)—before	23·6	9·2	23·0	16·3
(6) E.F.R. on calcium chloride (l./min.)—after	23·8	9·2	21·9	11·5
(7) E.F.R. on calcium chloride (l./min.)—difference	0·2	0	−1·1	−4·8
(8) E.F.R. on adrenaline (l./min.)—before	18·0	9·0	16·6	12·1
(9) E.F.R. on adrenaline (l./min.)—after	27·2	12·8	24·2	17·9
(10) E.F.R. on adrenaline (l./min.)—difference	9·2	3·8	7·6	5·8
(11) Difference in gain (x) of E.F.R. with calcium chloride compared with adrenaline (7)–(10)	−9·0	−3·8	−8·7	−10·6
(12) Cumulative total of (11)—i.e., Sx	−9·0	−12·8	−21·5	−32·1
(13) Cumulative total of the squares of (11)—i.e., Sx^2	81·00	95·44	171·13	283·49
(14) $Sx \div \sqrt{(Sx^2)}$	−1·00	−1·31	−1·64	−1·91

P.F.U.O.=Pulmonary fibrosis of unknown origin. A., C.B.=Asthma, chronic bronchitis.

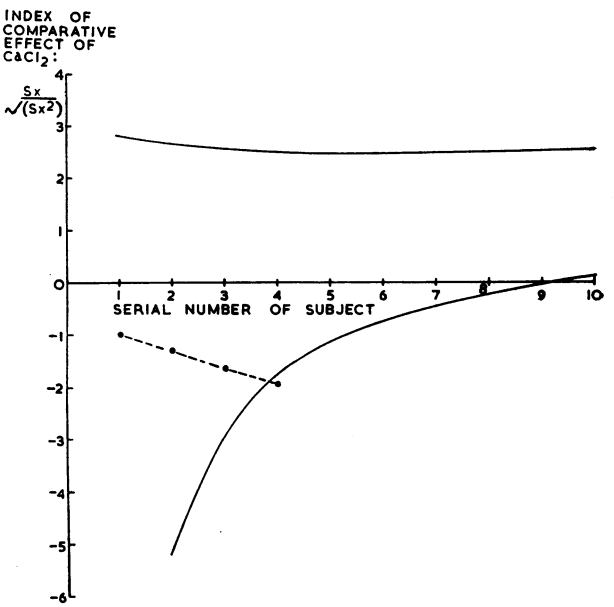

FIG. 2.—Result of first trial, with 20% calcium chloride.

after the fourth case had been tested, so that the absence of an advantage to calcium chloride over adrenaline could then be taken as established at the 1% level of significance.

One possible explanation of this disappointing result was that the dosage of calcium chloride was incorrect. Howell (1953) used a concentration of 10%, and it was evidently necessary to try to confirm his findings by an exact reproduction of his technique. Accordingly a fresh trial was started, using exactly the same procedure as before, except that calcium chloride was administered in a concentration of 10%. The results are shown in Table II and plotted in Fig. 3. They were exactly similar to the first set, and again the comparative inefficacy of calcium chloride was established significantly after four subjects had undergone tests.

TABLE II.—*Result of Second Trial, with 10% Calcium Chloride*

(1) Subject	5	6	7	8
(2) Sex	M	M	M	M
(3) Age (years)	57	30	41	57
(4) Condition	C.P.	B., C.B.	C.P.	C.P.
(5) E.F.R. on calcium chloride (l./min.)—before	29·9	39·3	16·9	38·5
(6) E.F.R. on calcium chloride (l./min.)—after	29·0	38·1	19·6	39·6
(7) E.F.R. on calcium chloride (l./min.)—difference	−0·9	−1·2	2·7	1·1
(8) E.F.R. on adrenaline (l./min.)—before	32·7	40·0	18·6	40·9
(9) E.F.R. on adrenaline (l./min.)—after	45·3	46·9	26·7	44·2
(10) E.F.R. on adrenaline (l./min.)—difference	12·6	6·9	8·1	3·3
(11) Difference in gain (x) of E.F.R. with calcium chloride compared with adrenaline: (7)–(10)	−13·5	−8·1	−5·4	−2·2
(12) Cumulative total of (11)—i.e., Sx	−13·5	−21·6	−27·0	−29·2
(13) Cumulative total of the squares of (11)—i.e., Sx^2	182·25	247·86	277·02	281·86
(14) $Sx \div \sqrt{(Sx^2)}$	−1·00	−1·37	−1·62	−1·74

C.P.=Complicated pneumoconiosis. B, C.B.=Bronchiectasis, chronic bronchitis.

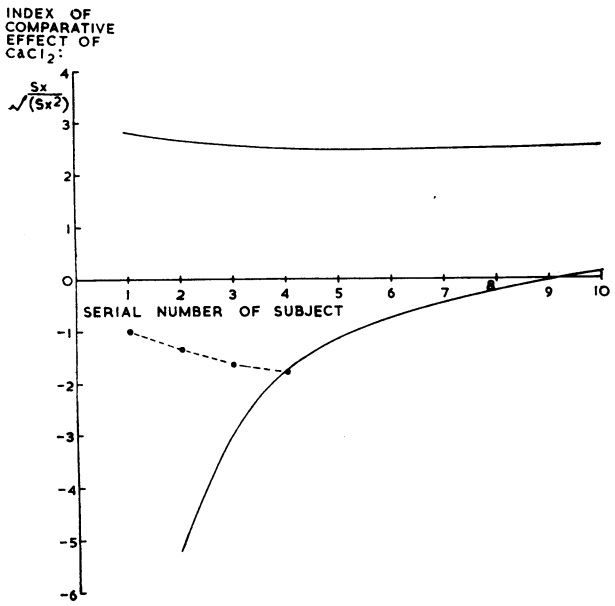

FIG. 3.—Result of second trial, with 10% calcium chloride.

The average effect of calcium chloride on all eight subjects was a loss of 0.5 litre per minute of E.F.R.; the average effect of adrenaline was a gain of 7.2 litres per minute.

Discussion

As the negative results of this trial of calcium chloride as a bronchodilator drug appear to conflict with recent reports, possible sources of error must be sought.

Despite the work of Charlier and Philippot (1949) and of Howell (1953), calcium chloride may not in fact be an effective bronchodilator drug in man, their findings having been the result of chance or lack of adequate controls in their experiments. There is some confusion in the literature about the possible action and modes of action of the calcium ion on the bronchial mucosa: Trendelenburg (1912) found it to have a bronchoconstrictor effect on perfusion of isolated segments of bronchi from cattle, whereas Zondek (1922) claimed a reverse effect; Rittman (1924) demonstrated a bronchodilator effect on isolated human bronchi, while Warnant (1930) showed a bronchoconstrictor effect on perfusion of the isolated lungs of guinea-pigs. More recently, Cauer and Neymann (1951), using inhalations of calcium in attempted prophylaxis and treatment of silicosis, reported some amelioration of dyspnoea, and Langen (1953) has suggested that, in man, calcium may augment the bronchodilator effect of an aminophylline derivative by virtue of its vasoconstrictor action on the bronchial mucosa.

The methods of administration of calcium chloride may have been unsuitable, but the usual technique of aerosol inhalation of both the 10% and 20% solutions was employed at a flow rate of 10 litres of oxygen per minute from a standard Collison inhaler, as Howell had previously noted that, in his opinion, calcium chloride was not so effective when given by means of a simple hand spray. As it had been our experience that most subjects with bronchial spasm responded to inhalations of 1/200 adrenaline given for 15 minutes, it was thought reasonable to assume that an alternative drug should have the same action (though possibly through a different mechanism) in a similar or shorter period of time. The inhalations were given randomly either in the morning or in the evening, so that the time of day would not influence the results.

It could be argued that one inhalation of calcium chloride in each subject was insufficient to produce a noticeable effect; Howell gave an inhalation of this substance thrice daily and claimed only that relief from wheezing was obtained after two days. While it is possible that the modes of action of adrenaline and calcium chloride are different, it is difficult to understand how any cumulative effect could result from three 15-minute inhalations of calcium chloride daily. Before our trial was started, calcium chloride had been administered thrice daily to four patients for several days, and in one instance for as long as two weeks. Only one patient said he felt better, while the remainder had to return to the more conventional bronchodilator agents.

The subjects included in this trial may not have been suitable, and the sample used may not have been representative of all cases of chronic bronchial spasm, but subjects with chronic bronchial spasm associated with diverse pulmonary pathology were intentionally included so that as fair a trial of calcium chloride as possible would be given.

Extraneous influences such as the even temperature of a hospital ward, chemotherapy for respiratory infections, other antispasmodics, and the influence (or charm) of the medical attendant were controlled as completely as possible, as most subjects with bronchial spasm are ever hopeful of some "new cure."

It has been pointed out (Fletcher, 1952) that a considerable observer-error exists in the interpretation of physical signs in the chest. The presence or absence of bronchial spasm can be detected on auscultation, but the degree of spasm present can be judged only by an arbitrary method of grading—for instance, mild, moderate, severe, or +, ++, +++. This is an unsatisfactory and, in our experience, an unreproducible method. In fact, we have found that there may be little change in the degree of bronchial spasm present, as judged by stethoscopy, before and after an inhalation of a known drug such as adrenaline, although its bronchodilator effect is detected by the E.F.R. test. Subjective sensations are also important, but are impossible to assess except comparatively, since most patients tend to feel better after any form of treatment if it is given with enthusiasm. Little reliance should be placed upon the patient's subjective sensations, and a trial of a new drug should be designed to test its effect on the soma rather than on the psyche. However, Table III shows the clinical

TABLE III.—*Clinical Assessment of Improvement of Bronchial Spasm*

	Serial No.	Patient's Opinion		Observer's Opinion	
		Adrenaline	$CaCl_2$	Adrenaline	$CaCl_2$
1st trial	1	+++	+	++	−
	2	++	+	−	−
	3	+++	−	++	−
	4	+	−	+	−
2nd trial	5	−	+	−	−
	6	++	−	++	−
	7	−	−	−	−
	8	+	+	−	−

assessments made before the E.F.R. measurements by both subject and observer, neither of whom knew which drug was being administered.

Rather than upon subjective impressions, the trial was based upon an objective test of the subjects' ventilatory ability. As one of the main effects of narrowing of the smaller bronchi is to reduce the maximum rate at which the lungs can be filled and emptied by air, it seems reasonable to take the maximum voluntary ventilation as assessed by the E.F.R. test as an index of the degree of bronchial spasm. It is our experience and Kennedy's (1953) that the E.F.R. test is a reliable and reproducible method of measuring bronchial spasm and its relief by a bronchodilator such as adrenaline. Kennedy, using a 15-minute inhalation of 1/1,000 adrenaline, found that this increase was of the order of 10% or more. In this trial, adrenaline 1/200 was used for the comparison, and its effect was to increase the E.F.R. by about 30%. Other methods of measuring the degree of bronchial spasm have been used in the past in the assessment of bronchodilator drugs; Charlier and Philippot used tidal-air tracings, while Robertson (1949) used the simple vital-capacity measurement in assessing the effect of isoprenaline ("neo-epinine"), but Gilson and Hugh-Jones (1949) have shown that this method, taken alone, can be an imperfect, and occasionally a misleading, index of the patient's ventilatory ability.

The numbers required to satisfy the statistical requirements of the trial were very small, and instinct tends to run counter to statistical theory in such circumstances, so that it is felt that what can safely be concluded is in fact less than theory suggests. However, the extremely consistent absence of effect shown by calcium chloride in eight subjects must reassure even the most non-statistical of sceptics that the result is a reliable one.

Some further remarks on the form of experiment carried out may be appropriate—namely, a sequential trial. This form of statistical testing was first developed by the late A. Wald in 1943, and was regarded as so important in effecting economies in the testing of military equipment that it was classed as "restricted" under the U.S. Espionage Act. As has been said, a sequential trial will require on the average a smaller sample than will any other form of trial, and this is clearly an important advantage in therapeutic trials, both from ethical considerations, which require that a decision shall be reached as soon as possible so that a new drug shall not be administered or withheld unnecessarily, and from considerations of economy. None the less, no application of the method to medicine can be traced in the literature, although it has been suggested by Armitage (1950), Bailey (1952), and Bross (1952). A detailed account of the method and its applications to medicine has recently been published by Armitage (1954).

The method undoubtedly has some disadvantages. It is necessary to be able to specify beforehand those critical values of the measured quantity which would be of material importance, and this can be difficult. Very often a trial is planned in the hope that a "clear-cut" answer will emerge, with no clear definition of what constitutes such an answer. This can lead in some cases to a trial giving statistically significant but materially insignificant results, and the difficulty of interpretation then appears. In the sequential method the difficulty must always be faced before the trial starts. The limits chosen in the present trial were carefully discussed beforehand with our colleagues in the unit, and considerable differences of opinion were encountered. Moreover, after the trial had concluded, the usefulness of the result established was the subject of further discussion and criticism, and it was suggested that different critical levels would have been more appropriate. A further difficulty is that in any one trial only one predetermined comparison can be tested for statistical significance; the significance of any other results must remain uncertain. This applies, for example, to the observed average effects on the E.F.R. of the two drugs, which cannot be tested against a standard error. It also applies to the results of the subjects' and observers' impressions.

On the whole the advantages of the technique seem undoubtedly to outweigh its disadvantages, and it is the intention of this unit to carry out further trials of drugs in a similar manner. One important change will be made, however. The present trial was incapable of ending with the conclusion that calcium chloride was significantly *worse* than adrenaline, and after the trial was concluded it was pointed out to one of us by Dr. P. Armitage, of the M.R.C. Statistical Research Unit, that it would be useful to be able to end with a firm decision in favour of either of the two treatments, besides the possibility of suspending judgment. The technical procedure for such a two-sided trial is no more difficult than for the single-sided one; it is described by Rushton (1952) and, with direct application to clinical trials, by Armitage (1954).

Summary

Inhalations of calcium chloride as a means of relieving bronchial spasm were tested against adrenaline in a controlled trial. The effect of the inhalations was compared with the effect of inhalations of adrenaline. The subjects all suffered from chronic bronchial spasm of diverse aetiology.

The expiratory flow rate of the subjects decreased on the average by 0.5 litre per minute after inhaling calcium chloride, whereas it increased by 7.2 litres per minute after adrenaline. It could be concluded, with but one chance in a hundred of error, that calcium chloride is not appreciably better than adrenaline in relieving bronchial spasm.

This investigation used, for the first time in clinical research, the statistical technique of sequential analysis, which enables clear-cut conclusions to be reached with, on the average, the smallest number of experimental subjects. Only eight subjects were needed to substantiate the conclusion drawn.

Some of the subjects in this trial were under the care of Dr. D. A. Williams, and we would like to thank him for allowing us to include them. We thank also our colleagues in the Pneumoconiosis Research Unit for their advice in the planning of the trial.

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Discussing "Expert Medical Testimony" (*Amer. J. clin. Path.*, 1954, **24**, 1149), Dr. Bernhard Steinberg writes: "Obviously, it is impossible to be an expert in all fields of medicine. Yet the court and attorneys, by custom and law, expect the impossible. Unfortunately, the doctor usually acquiesces. Under usual circumstances, the average physician is careful with any medical allegations he may make. Not so in the courtroom. There is something about the scene that makes the doctor throw all caution to the winds. He becomes an expert in all things. The result is a fantastic mass of evidence. Some of it is outright inaccurate and much of it without benefit of pre-existing fact."