

worthy of trial in the treatment of experimentally induced poliomyelitis.

## SUMMARY

A new experimental technique has been used for studying the fate of particles injected into the subarachnoid space of newborn rats.

The implications which the results of these experiments have on the pathology and therapy of poliomyelitis are discussed.

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## THE TREATMENT OF INTERMITTENT CLAUDICATION WITH VITAMIN E

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The suggestion that administration of vitamin E is beneficial in peripheral arterial disease was originally made by Shute et al. (1948). Its use has been advised in the treatment both of angina pectoris and of intermittent claudication. In angina the earlier favourable claims (Vogelsang and Shute 1946, Vogelsang et al. 1947) were not confirmed by the more carefully controlled studies of later observers (Makinson et al. 1948, Donegan et al. 1949, Ravin and Katz 1949). On the other hand the administration of synthetic vitamin E in cases of intermittent claudication of moderate severity has been strongly advocated by Boyd et al. (1949), Ratcliffe (1949), and Boyd (1951), and as a result this drug is now widely prescribed.

If vitamin E acts by expediting the recanalisation of occluded arteries or the development of collateral arteries in cases of intermittent claudication, it is difficult to understand why it should not be equally effective in angina pectoris when this is due to occlusive coronary arterial disease. In a small series of cases of intermittent claudication Hamilton and Wilson (1952) found that synthetic vitamin E had no effect on exercise tolerance, which was measured at short intervals throughout the period of treatment.

In view of this conflict of evidence the action of vitamin E in intermittent claudication has been further investigated in a larger series of cases. On this occasion the natural product, as recommended by Shute et al. (1948), rather than the synthetic preparation, has been used.

TABLE I—SIMILARITY OF VITAMIN-E AND CONTROL GROUPS (Table shows mean values with their standard errors for each group)

	Vitamin E (20 cases). Mean $\pm$ S.E.	Control (21 cases). Mean $\pm$ S.E.
Age (years)	54.8 $\pm$ 1.8	55.9 $\pm$ 1.8
Duration of illness (years)	3.6 $\pm$ 0.5	3.3 $\pm$ 0.5
<i>Exercise-tolerance tests:</i>		
Average number of circuits	19.0 $\pm$ 1.6	18.1 $\pm$ 1.6
Average duration of pain after exercise (sec.)	42.0 $\pm$ 5.9	55.7 $\pm$ 5.8
Average time per circuit (sec.)	5.6 $\pm$ 0.2	6.5 $\pm$ 0.2

## METHODS

41 patients have been studied. All had typical intermittent claudication of the calf muscles due to occlusive arterial disease. In only 1 patient was the arterial disease the result of thrombo-angiitis obliterans. In the remaining 40 cases the arterial lesion was assumed to be atheromatous. None of the patients had diabetes. Exercise-tolerance tests were made, so far as possible, at fortnightly intervals by walking over steps (Hamilton and Wilson 1952). The patient halted as soon as the pain in the legs developed. The number of circuits over the steps and the duration of the exercise were recorded, and the rate of walking was calculated. The duration of the pain after stopping was also measured. A full clinical examination was made before and after the course of treatment, and the patient's opinion about any change in his capacity to walk was ascertained.

Natural vitamin E was dispensed in gelatin-coated capsules, the specification for their preparation being that "the d. alpha tocopheryl acetate shall be prepared from vegetable oils and each capsule shall contain Type VI concentrate, equivalent to 75 mgm. d.l. tocopheryl acetate." Similar capsules were filled with arachis oil, and these control preparations were identical in size and colour with the vitamin-E capsules so that they could not be distinguished in any respect by either the investigators or the patients. During the twelve-week period of treatment two capsules were taken thrice daily. The dose of vitamin E was accordingly 450 I.U. per day.

Numbered sets of bottles containing either vitamin E or arachis oil were prepared in the Statistical Research Unit of the Medical Research Council; the order of administration of the two treatments was based upon a list randomly constructed in the unit and held there. After a preliminary period of observation of at least a month, the patient was allotted a number and took the similarly numbered capsules continuously for twelve weeks. The investigations were continued for a further month after the period of treatment. The nature of the treatment in individual cases was not revealed to the investigators until after the whole trial was completed.

## COMPARABILITY OF GROUPS BEFORE TREATMENT

As the treatment allocated to each patient was determined randomly, a careful comparison of those receiving vitamin E and those receiving arachis oil is essential. This has been based on the observations made before treatment, and the results are shown in table I. The mean age and the mean duration of the illness are similar in both groups. For each patient the number of circuits walked before the onset of pain was recorded at each visit before treatment, and from these observations the

TABLE II—SIMILARITY OF VITAMIN-E AND CONTROL GROUPS BY BOYD CLASSIFICATIONS

Grade	Vitamin E	Control	Total
II	11	9	20
III	9	12	21
Total	20	21	41

TABLE III—COMPARISON OF VITAMIN-E AND CONTROL GROUPS ACCORDING TO PATIENT'S ASSESSMENT OF RESULT OF TREATMENT

Result	Vitamin E	Control	Total
Improved ..	8	6	14
No change ..	12	14	26
Deteriorated ..	0	1	1
Total ..	20	21	41

patient's individual average number of circuits was calculated. The means of these individual averages were then computed in the two groups, and they are shown in table I to be quite close. Similarly, for each patient the average duration of pain after stopping exercise was determined. The mean value was shorter in the vitamin-E group but not significantly so. The two groups do, however, differ significantly in the average time taken per circuit. The vitamin-E group take, on the average, 5.6 seconds per circuit, against 6.5 seconds for the control group; the difference of 0.9 has a standard error of 0.3, and such a difference, or a larger one, would be expected to occur purely by chance only about once in fifty times. Thus the random allocation has led to rather more of the initially brisker walkers being chosen for the vitamin-E than for the arachis-oil treatment. This means that any apparently marked effect of treatment in favour of vitamin E must be examined carefully to see whether it may have resulted from the initial disparity in walking speeds between the groups.

The distribution of cases, as classified by the method of Boyd et al. (1949), is shown in table II. There is no conspicuous difference between the groups, but again the vitamin-E group contains a slightly higher proportion (55%) of grade-II (less severe) cases than the control group (43%).

TABLE IV—INCREASE IN AVERAGE NUMBER OF CIRCUITS DURING WHOLE TREATMENT PERIOD COMPARED WITH PRE-TREATMENT PERIOD

Group	No. of cases	Average no. of circuits		Mean increase $\pm$ standard error
		Pre-treatment	During treatment	
Vitamin E ..	20	19.02	20.66	1.64 $\pm$ 0.86
Control ..	21	18.12	19.04	0.92 $\pm$ 0.84
Difference 0.72 $\pm$ 1.20				

## RESULTS OF TREATMENT

Various measures of the improvement shown by the two groups during or after the period of treatment are summarised in tables III-VII. The patients' own assessments of the effect of treatment are tabulated in table III. There is clearly no appreciable difference between the two groups. These subjective assessments of the patients are more optimistic than the objective ones presented in the following tables. Throughout the period of treatment the observers could not distinguish between patients given vitamin E and those given arachis oil either by the patients' statements or by clinical examination.

For each patient the average number of circuits walked in the pre-treatment period (the measure used in table I) was subtracted from the average number walked during the whole of the treatment period. This difference provides a measure of the patient's improvement in exercise tolerance during the treatment period. Table IV shows the mean increase that took place in each of the groups, with its standard error. It will be seen that in neither group was there any substantial improvement in performance. The vitamin-E group shows a slightly greater increase (0.72 circuits) compared with the control group, but the difference is not significant.

Very similar results are shown in table V, where the pre-treatment averages are contrasted with those prevailing during the last month of treatment. The vitamin-E group again shows a slight, but non-significant, improvement over the control group. The control group here was reduced to 19 patients because 2 of the 21 did not visit the clinic during this part of the treatment period.

The possibility that the treatment took effect after the conclusion of the three months' treatment is examined in table VI. Here, for each patient, the pre-treatment average number of circuits is subtracted from the average number achieved during the three months following the end of treatment. The post-treatment visits were rather irregular, but all the patients except 1 in each group had at least one visit during this period. Once again the effect shown is small, and there is no significant difference between the groups.

TABLE V—INCREASE IN AVERAGE NUMBER OF CIRCUITS DURING LAST MONTH OF TREATMENT COMPARED WITH PRE-TREATMENT PERIOD

Group	No. of cases	Average no. of circuits		Mean increase $\pm$ standard error
		Pre-treatment	Last month of treatment	
Vitamin E ..	20	19.02	20.86	1.84 $\pm$ 0.96
Control ..	19	17.81	19.03	1.22 $\pm$ 0.99
Difference 0.62 $\pm$ 1.38				

The duration of pain that was experienced by each patient after his exercise-tolerance test is analysed in table VII. The average duration experienced by the patient in the pre-treatment period (as used in table I) was subtracted from the corresponding average observed during the treatment period. The difference between these two values was then expressed as a percentage of the pre-treatment values to reveal the relative improvement (or deterioration) in the patient. For instance, in the pre-treatment period a patient may, on the average of his observations, have experienced pain for 40 seconds. During treatment his average duration may have become 30 seconds, and this is represented by an improvement of 10 seconds in 40 (25%). The means, over the two groups of these individual percentage changes are given in table VII. In the whole period of treatment the vitamin-E group shows a small and non-significant reduction of 9.7% in duration of pain, while the control group shows a small and non-significant increase of 8.3%. The difference between these two values (18%) has a standard error of 7.0% and is therefore technically significant.

It is essential to determine whether this significant result in favour of the vitamin-E group is due merely to the disparity between the groups which has been noted earlier. Analysis within the two groups shows that it was patients who took the longest time over a circuit in the pre-treatment period who tended also, on the average, to show subsequently the least improvement

TABLE VI—INCREASE IN AVERAGE NUMBER OF CIRCUITS DURING FIRST THREE MONTHS AFTER TREATMENT COMPARED WITH PRE-TREATMENT PERIOD

Group	No. of cases	Average no. of circuits		Mean increase $\pm$ standard error
		Pre-treatment	First three months after treatment	
Vitamin E ..	19	18.88	19.85	0.97 $\pm$ 1.01
Control ..	20	18.32	19.82	1.50 $\pm$ 0.99
Difference -0.53 $\pm$ 1.41				

in their duration of pain—i.e., comparing the pre-treatment and treatment periods. Thus, for each extra second in the original time that a patient took per circuit there was, on the average, a 6.7% increase in the duration of pain observed during treatment (the regression coefficient of 6.7 has a s.e. of 3.2 and is therefore significant at the 0.05 level). It has already been shown (table I) that the vitamin-E group has a mean time per circuit of 5.6 seconds, compared with 6.5 seconds for the controls. This difference, which to two decimal places is 0.85 seconds, would alone be expected to cause a subsequent difference between the two groups in the percentage change in duration of pain—i.e.,  $(0.85)(6.7) = 5.7\%$ . The observed difference of 18.0% in table VII should therefore be reduced by 5.7 to give the correct value, 12.3%. This corrected value still favours the vitamin-E group, but it has a standard error of 7.2% and is thus not technically significant, although it is sufficiently nearly so to require further consideration.

These observed changes in the duration of pain during the course of treatment were also analysed in relation to the treatment schedule. Table VII shows that the relative improvement of the vitamin-E group, compared with the deterioration of the control group, was slightly more distinct in the first two months' treatment than later. The corrected difference of 15.5% just reaches significance at the 0.05 level. In the third month of treatment and in the post-treatment period the differences, on the other hand, are not significant. In both groups in the post-treatment period the changes from the pre-treatment level are quite small and unimpressive.

The rather small numbers of patients available in this trial may have led to moderate effects of vitamin-E

TABLE VII—CHANGE IN AVERAGE DURATION OF PAIN DURING AND AFTER TREATMENT AS PERCENTAGE OF PRE-TREATMENT AVERAGE

Period	No. of cases	Mean $\pm$ standard error	After correction for time/circuit	Regression of percentage increase in pain on time/circuit
0-12 weeks' treatment	20 21	Vit. E $-9.7 \pm 5.0$	$-12.3 \pm 7.2$	$6.7 \pm 3.1$
		Control $+8.3 \pm 4.9$		
		Difference $-18.0 \pm 7.0$		
0-8 weeks' treatment	20 21	Vit. E $-10.2 \pm 5.4$	$-15.5 \pm 7.7$	$7.5 \pm 3.3$
		Control $+11.7 \pm 5.3$		
		Difference $-21.9 \pm 7.5$		
8-12 weeks' treatment	20 19	Vit. E $-10.0 \pm 5.0$	$-11.4 \pm 7.6$	$4.4 \pm 3.5$
		Control $+4.9 \pm 5.1$		
		Difference $-14.9 \pm 7.1$		
Post-treatment period	19 20	Vit. E $-8.5 \pm 6.5$	$-12.0 \pm 9.9$	$1.9 \pm 4.3$
		Control $+5.2 \pm 6.3$		
		Difference $-13.7 \pm 9.1$		

treatment not being detected, owing to the considerable variability between patients. It is therefore of some interest to record the probable upper and lower limits to the effects of vitamin-E treatment as revealed by these controlled observations. These points are "95% confidence limits," between which the true mean effect is likely to lie. They are obtained by taking the observed differences in means given in tables IV-VII, and adding to, or subtracting from, them "t" times the standard error. (The factor t is the 0.05 level of the t-distribution on N-2 degrees of freedom, where N is the total number of patients used in the comparison. For a comparison using all the patients, as in table IV, N = 41 and t = 2.02.) The odds are 19 to 1 that the two limits include the true mean value for the type of patient used in this trial. The limits are given in table VIII. Those for increases

TABLE VIII—95% CONFIDENCE LIMITS FOR MEAN EFFECTS OF VITAMIN E IN COMPARISON WITH CONTROLS

Comparison	Limits
Table IV—Average increase in circuits during treatment	- 1.7 to + 3.1 circuits
Table V—Average increase in circuits during last month of treatment	- 2.2 to + 3.4 circuits
Table VI—Average increase in circuits after treatment	- 3.4 to + 2.3 circuits
Table VII—Average change in duration of pain, as percentage of pre-treatment level	- 26.8% to + 2.3%

in exercise tolerance probably exaggerate the possible beneficial effect of vitamin E, owing to the original disparity of the groups. In the calculation of limits for the percentage change in duration of pain the corrected value of table VII has been used.

#### DISCUSSION

In determining the results of treatment reliance has been placed on the statements of the patients, the clinical examination of the lower limbs, and the performance of exercise-tolerance tests. Neither of the first two indicated that vitamin E was in any way superior to arachis oil. In the exercise-tolerance tests treatment produced no marked or technically significant difference between the two groups as regards the distance that could be walked before the onset of pain. The only difference of any substance found between the two groups was in the duration of pain after exercise. During the whole period of treatment this increased by + 8.3%, on the average, in the controls and decreased by - 9.7% in the vitamin-E group. The difference between the two figures was shown to be significant. The statistical analysis of the results also showed that the duration of pain after exercise tended to become significantly greater in the slow walkers in both treatment groups as the trial proceeded. These slow walkers were initially rather more numerous in the control group; and, if a correction is made for this discrepancy, the difference between the two groups is no longer technically significant, though it continues to favour slightly the patients on vitamin E.

From the clinical point of view, however, too much importance should not be attached to these results, because the assessment of the duration of a pain that gradually fades away is notoriously difficult, and there was considerable variability in these measurements even in the same patient. It will be noted that the changes from the pre-treatment values are small in each of the two groups, and they are of significance only because they are in opposite directions. A more detailed analysis of the results showed that the difference between the two groups was greatest during the first two months' treatment but was non-significant during the third month and the period immediately after treatment. This is the reverse of what might be expected if the changes were the result of vitamin-E therapy.

The results of this investigation have shown that the beneficial effect of vitamin E in the treatment of these cases of intermittent claudication was not appreciably greater than that of arachis oil, and the earlier favourable reports of Boyd et al. (1949) have not been confirmed. Our failure to demonstrate improvement could be due (1) to the methods of assessment being insufficiently exact, with the result that a real improvement was overlooked, or (2) to the wrong type of patient being treated. In our exercise-tolerance tests the patients walked over steps, whereas in Boyd's series they walked on the level, either along hospital corridors or on a moving platform. The two methods, however, are fundamentally similar because they both measure the amount of exercise required to produce pain. A true improvement is reflected in an increase in the amount of exercise that can be taken before the onset of pain and in a decrease

in the duration of pain in spite of the greater effort (Hamilton and Wilson 1952). If the treatment were beneficial, a definite improvement in the vitamin-E group over the controls should have been revealed by this method of performing exercise-tolerance tests.

Patients with intermittent claudication of all degrees of severity have been studied, though Boyd (1951) has stated that only the less seriously affected (his grades I and II) respond to vitamin E. With the more severe exercise of walking over steps the pain, once developed, persists in all patients, and with this test none fall into Boyd's grade I. Our grades II and III thus include several cases that would have fallen into lower groups if the tests had been performed by walking on the level. There is thus no doubt that the series contained in both control and treated groups many of the milder cases reputed to respond to vitamin E.

These observations and considerations indicate clearly that vitamin E is of no appreciable benefit to patients with intermittent claudication. The previous favourable reports have not included details of any adequate control studies. In investigations of intermittent claudication reliance has always to be placed on an endpoint determined by the patient's assessment of his pain. It has previously been demonstrated that this may be modified by any action involving therapeutic suggestion. In these circumstances the trial of any drug must be most rigorously controlled. Frequent exercise-tolerance tests are essential before, during, and after the period of treatment, preferably at fortnightly intervals. Finally, the trial should be conducted on the "double blind" principle, and before any drug is claimed to be effective it must be shown to be so when both patient and observer are unaware of the nature of the treatment.

#### SUMMARY

The effect of natural vitamin E in intermittent claudication was investigated in 41 patients, divided at random into control and treatment groups. The controls received capsules containing arachis oil, and the treatment group received capsules identical in appearance but containing vitamin E. The daily dose was 450 I.U., the equivalent of 450 mg. of tocopherol, and was continued for twelve weeks.

Until the whole trial was completed the clinical observers (and the patients) were unaware of the treatment given in any particular case. The assessment of the effect of therapy was based on the patient's opinion, the clinical examination, and the results of exercise-tolerance tests.

No appreciable difference was found between the response of the two groups.

It is concluded that vitamin E is of no value in the treatment of intermittent claudication.

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## AUTOMATIC FRACTIONAL ANALYSIS OF EXPIRED AIR AS A CLINICAL TEST

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Roelsen (1939) demonstrated that in emphysematous patients, more than in normal people, the composition of the expired air varied with the volume expired. His findings have been confirmed by Marshall, Bates, and Christie (1952), who interpret them as evidence of poor spatial correlation between ventilation and perfusion in emphysematous lungs. The possibility thus arises of using fractional analysis of expired air as a clinical test for emphysema. However, because of the labour entailed by the analysis of multiple samples of gas, the methods of the previous workers are hardly suitable for routine clinical use.

We describe here the use of a commercial analyser for continuous recording of the carbon dioxide content of respired air. The abnormal pattern is easily recognised.

and, since repeated observations are conveniently made, the method lends itself to a study of the influence of relevant factors. A somewhat similar procedure, using a specially constructed instrument, was described by Dubois et al.

(1952), who were, however, concerned with normal rather than pathological respiration.

#### METHODS

The patient breathes into a Benedict spirometer fitted with a carbon dioxide absorber. Attached to the wheel of the spirometer is a potentiometer which gives an electrical record of the amount of air in the bell at any moment. A side tube to the mouthpiece is connected by a short length of rubber tubing to the analyser.\* Air is drawn, at a rate of about 30 ml. per second, from the mouthpiece through the analyser by a small electric pump and is then returned to the spirometer bell. In this way the respired gases are continuously sampled without disturbing the registration of their volume. (fig. 1).

The analyser's electrical system uses amplitude modulation of a 6-cycle-per-sec. carrier, rectified and smoothed to give a response time of several seconds. As a much quicker response was required, the unrectified signal was taken out and rectified, without smoothing, by an external circuit. With this arrangement the response to sudden changes of gas mixture is complete in about one-third of a second.

Sometimes, in addition, intrathoracic pressure and trunk expansion were recorded by the method of Dornhorst and Leathart (1952).

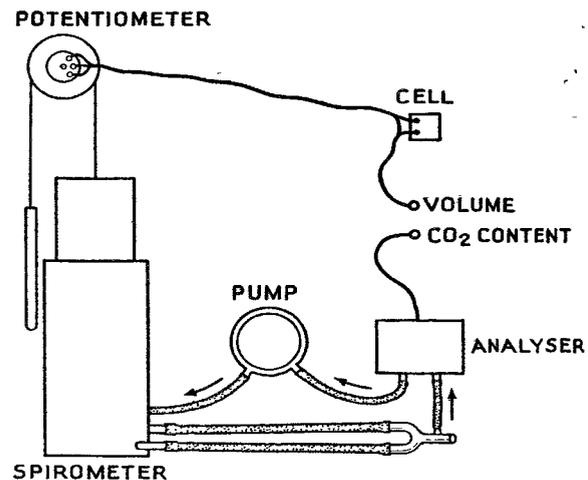


Fig. 1—Arrangement of spirometer and carbon dioxide analyser.

\* Infra Red Development Co. Ltd. Type S.C.