Eur Respir J, 1994, 7, 1090–1095
DOI: 10.1183/09031936.94.07061090
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European Respiratory Journal
ISSN 0903 - 1936

Autonomic nerve dysfunction in COPD as assessed by the acetylcholine sweat-spot test

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Autonomic nerve dysfunction in COPD as assessed by the acetylcholine sweat-spot test. A.G. Stewart, F. Marsh, J.C. Waterhouse, P. Howard. ©ERS Journals Ltd 1994.

ABSTRACT: Patients with hypoxic chronic obstructive pulmonary disease (COPD) have evidence of a subclinical parasympathetic autonomic neuropathy, with apparent preservation of sympathetic function. However, these cardiovascular-respiratory tests might have been biased by concomitant chest disease, the acetylcholine sweat-spot test avoids this bias. This sweat-spot test assesses sympathetic nerve function, it relies upon the fact that denervated sweat glands do not produce sweat.

35 patients with hypoxaemic COPD and seven age matched normal subjects were studied. Following intradermal injection of 0.1 of 1% acetylcholine into the dorsum of the feet, the number of sweatglands able to respond in a given surface area was recorded. Cardiovascular autonomic nerve function, arterial oxygen and carbon dioxide tensions, lung function and cigarette consumption were also recorded.

The acetylcholine sweat-spot test was highly repeatable in eight COPD patients, no person with normal or frankly abnormal function being wrongly assigned. The age matched control subjects had normal acetylcholine sweat-spot scores and cardiovascular autonomic tests. The acetylcholine sweat-spot test was abnormal in 24 patients, borderline in 8 and normal in 3 patients. The abnormal sweat-spot test group had significant worse FEV_1, arterial blood gases and autonomic function. The acetylcholine sweat-spot score correlated with the severity of arterial hypoxaemia ($r=-0.78$, $p<0.001$) and with the parasympathetic cardiovascular tests ($r=0.80$, $p<0.001$).

In conclusion, patients with hypoxaemic COPD have a parasympathetic cardiovascular and a peripheral sympathetic autonomic neuropathy. The acetylcholine sweat-spot test is repeatable, easy to perform and a sensitive indicator for autonomic dysfunction in breathless individuals with COPD.

Eur Respir J., 1994, 7, 1090–1095.

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Keywords: Acetylcholine sweat-spot test
autonomic nerve function
chronic obstructive pulmonary disease

Received: August 11 1993
Accepted after revision January 4 1994

Evidence has accumulated suggesting that intraneural hypoxaemia provides an important component of diabetic neuropathy [1–3], and may account for some of the pathological similarities seen in the neuropathies of diabetes mellitus and hypoxaemic chronic obstructive pulmonary disease (COPD) [4]. We have recently shown that patients with hypoxaemic COPD have evidence of a subclinical cardiovascular autonomic neuropathy [5].

The reflexes assessed in these cardiovascular autonomic tests are extremely complex and do not determine the site of dysfunction. The abnormality may occur in stimulus reception, afferent nerve conduction, central processing, efferent nerve conduction, motor end-plate or end-organ (*e.g.* heart) response. Furthermore, the heart rate response to Valsalva manoeuvre and deep breathing involve respiratory afferent limbs. It is, therefore, conceivable that pulmonary disease and stimulus reception rather than neuropathy account for some of the abnormalities that we reported previously [5].

Our study of 96 patients with COPD showed clear evidence for parasympathetic dysfunction [5], but failed to show any evidence of sympathetic dysfunction. However, this might have been due to the relative crudeness of tests, based upon blood pressure responses to standing and sustained hand grip.

In an attempt to confirm our previous findings and to further investigate the potential sites of the autonomic lesion, we performed acetylcholine sweat-spot tests on patients with hypoxaemic COPD. This test has been shown to be a sensitive test of sympathetic nerve function [6, 7]; it relies upon the principle that denervated sweat glands lose their ability to produce sweat [8]. The acetylcholine causes sweating, either by diffusion or a local axonal reflex. It is not a complex reflex, nor does it require central processing [7, 8].

On the principle that in a general neuropathy the longest nerves (the sympathetic nerves to the skin of the feet) would be damaged first, we performed the acetylcholine sweat-spot test on the feet of patients with hypoxaemic COPD, to look for signs of a sympathetic autonomic neuropathy.

Subjects and methods

Thirty five subjects with a clinical and spirometric diagnosis of COPD were recruited from the chest clinic, and compared to seven age-matched symptom-free subjects recruited from hospital staff and patient's spouses. The group was selected to ensure that no patient gave a history, had physical signs or investigational evidence for diabetes mellitus, hypertension, cerebrovascular accidents, Shy-Drager syndrome, rheumatoid arthritis, hyponatraemia, carcinoma, renal failure, liver disease or peripheral vascular disease.

Subjects on medications likely to interfere with the tests, such as vasodilators, angiotensin-converting enzyme inhibitors and antihypertensive agents, were also excluded. Those patients on diuretics stopped taking them for three days before the test. Theophyllines and oral β_2-agonists were discontinued for 24 h, and ipratropium bromide was not used on the day of the test. Toiletries affecting sweat release were prohibited.

After informed consent, a brief history and clinical examination was performed. Spirometry, and arterial blood gas tensions were measured.

Autonomic neuropathy tests

Heart rate response to a Valsalva manoeuvre. The seated patient took a moderate inspiration, then blew into a mouthpiece attached to a sphygmomanometer, and maintained a pressure of 40 mmHg for 15 s. The patient then breathed normally for 1 min. The Valsalva ratio was calculated as the maximum heart rate recorded during the Valsalva manoeuvre, divided by the slowest rate recorded on release. An average of three readings was recorded. A value of <1.11 is abnormal, while values of >1.20 are normal [9].

Heart rate response to deep breathing. With the patient seated, the heart rate was recorded during six deep breath cycles in a 1 min period. The inspiratory-expiratory (I-E) difference was the average of the maximum differences in heart rate during each breath. Patients with an autonomic neuropathy lose this heart rate variability. A heart rate difference of <8 beats·min^{-1} is abnormal, values of >10 are normal [10].

Heart rate and blood pressure response to standing. After lying supine for 3 min, the systolic blood pressure was recorded. The patient then stood up, unaided. The heart rate response was recorded and the systolic blood pressure taken 10–15 s after attaining the upright position. A systolic fall >30 mmHg is abnormal, a fall of <10 mmHg is considered normal [9].

The 30:15 ratio. This ratio is the longest R-R interval around beat 30 divided by the shortest interval at beat 15. Patients with autonomic neuropathy have a heart rate which continues to rise throughout this observed time, and thus have a ratio of ≤1. The average of two recordings was taken. A value of <1.01 is abnormal, and values >1.04 are normal [9].

The four tests were scored, 0 if normal, 1 if borderline, and 2 if abnormal, giving a maximum score of 8.

Acetylcholine sweat-spot test. This test was performed on the same day, using the method of RYDER and coworkers [6, 7], in supine rested subjects. Briefly, an area 36×24 mm was marked out on the upper surface of each foot. The area was painted with 2% iodine and, when dry, coated with a maize starch solution. One tenth of a millilitre of 1% acetylcholine was injected intradermally in the centre of the marked area. This caused innervated sweat glands to release sweat, producing a blue spot over each sweat gland.

The area was photographed after 6 min [6] with a fixed object distance, such that magnification was always 1:1 and a 10× enlargement was produced. A grid of 64 squares (2.5×2.5 cm) was placed over the enlarged photograph with the injection site at the centre; the central four squares were excluded. In the remaining 60 squares, the number of squares with less than six sweat gland spots was counted.

None or one such square is normal, 5 or more squares is consistent with a sympathetic autonomic neuropathy in patients with diabetes mellitus [6, 7]. Tests with 2–4 squares with less than 6 sweat-spots were defined as borderline. In eight patients the tests were repeated after an interval of one month. These patients had results covering the normal to just abnormal range, to determine whether or not the repeatability allowed us to discriminate between those with normal and abnormal sympathetic function.

Statistics

Repeatability was assessed by the method of BLAND and ALTMAN [11]. Spearman rank correlation coefficients were used to compare results with spirometry, arterial blood gases and cigarette history. The control group, those COPD patients with an abnormal acetylcholine sweat-spot test, and those COPD patients with normal or borderline sweat-spot results were compared by analysis of variance (ANOVA).

Results

The acetylcholine sweat-spot test was easy to perform. No patients experienced more than a slight discomfort from the needle prick, probably due to the presence of iodine. No practical problems were encountered, other

Table 1. – Number of squares with less than six sweat-spots in 8 COPD patients who had the test repeated after one month

Patient	ES	KA	JS	PB	CHP	SR	DV	EST
Test 1	3	2	8	4	10	11	0	3
Test 2	4	3	10	4	10	10	0	4

COPD: chronic obstructive pulmonary disease.

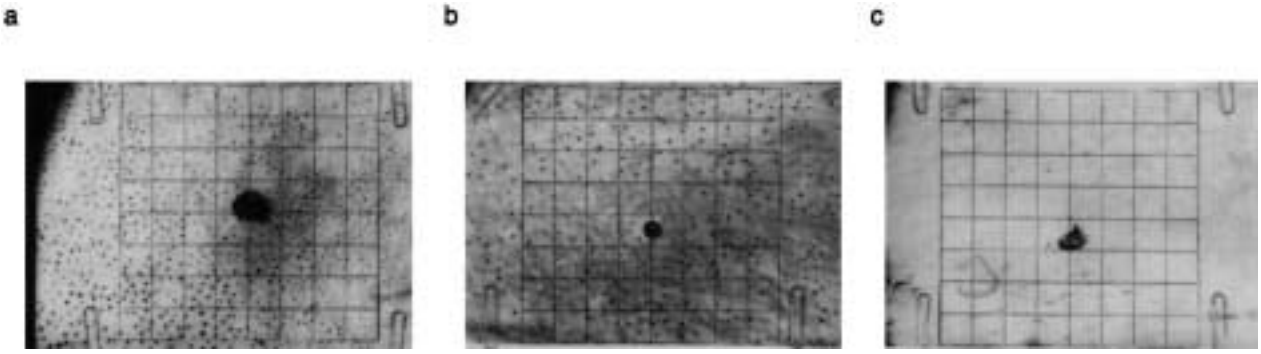

Fig 1. – a) COPD patient (KA), aged 66 yrs, sweat-spot score 2/60, Pa_{O_2} 10.3 kPa. b) COPD patient (HH), aged 66 yrs, sweat-spot score 39/60, Pa_{O_2} 7.1 kPa. c) COPD patient (AW) aged 71 yrs, sweat-spot score 60/60, Pa_{O_2} 5.9 kPa. COPD: chronic obstructive pulmonary disease; Pa_{O_2}: arterial oxygen tension.

Table 2. – Characteristics of control subjects and COPD patients grouped according to sweat-spot score

Parameter	Controls n=7	COPD with sweat-spot score ≤4 n=11†	COPD with sweat-spot score >4 n=24
Age yrs	66 (7)	63 (7)	67 (6)
Sex M/F	4/3	8/3	12/12
Pa_{O_2} kPa	11.0 (0.3)	9.4 (1.2)	7.3 (0.8)***
Pa_{CO_2} kPa	5.3 (0.1)	5.8 (1.1)	6.2 (1.1)
FEV_1 *l*	2.7 (0.6)	1.6 (0.6)	0.8 (0.3)***
FVC *l*	3.9 (0.5)	3.3 (0.9)	2.0 (0.6)**
Smoking pack-yrs	10.7 (9.8)	14.4 (9.9)	26 (18)
Autonomic score	0.7 (0.9)	0.6 (1.0)	4.0 (2.0)**
Valsalva ratio	1.41 (0.14)	1.41 (0.14)	1.16 (0.09)**
I-E difference	12.5 (6.0)	13.0 (4.3)	9.5 (5.9)
30:15 ratio	1.14 (0.09)	1.10 (0.04)	1.04 (0.04)**
BP fall mmHg	7.3 (3.5)	5.5 (6.2)	13.6 (8.1)*

Data are presented as mean and SD in parenthesis. †: 3 normal, 8 borderline sweat-spot scores; COPD: chronic obstructive pulmonary disease; Pa_{O_2}: arterial oxygen tension; Pa_{CO_2}: arterial carbon dioxide tension; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; I-E: inspiratory-expiratory; 30:15 ratio: longest R-R interval around beat 30 divided by the shortest interval at beat 15; BP: systemic blood pressure. Difference between groups by analysis of variance (ANOVA), *: $p<0.05$; **: $p<0.01$; **: $p<0.001$.

than ensuring adequate focusing of the camera. It was important to ensure that the starch solution was regularly replaced, otherwise the quality of the photographs declined.

The tests were reproducible in eight patients, whose sweat-spot scores ranged from 0–11 abnormal squares (table 1). The means of the two sets of readings were 5.1 and 5.6, with a standard error of difference of 0.35 and a coefficient of repeatability of 0.9 squares. Such a level of repeatability is acceptable. No patient would be misassigned to the normal or abnormal autonomic function groups. However, patients with borderline results (2–4 squares with less than six sweat-spots) may or may not have evidence for autonomic dysfunction.

Typical responses are shown in figure 1. Patient characteristics and results are shown in table 2.

Six of the normal subjects had 1 or less abnormal squares (with less than 6 sweat-spots), the other subject had 2 squares (a borderline result). They all had normal cardiovascular autonomic function. However, only 3 patients with COPD had normal sweat-spot scores; two of those being normoxic, the other having a Pa_{O_2} of 7.5 kPa. A further 8 had borderline tests. All 11 patients had normal cardiovascular autonomic scores. The remaining 24 patients had frankly abnormal acetylcholine sweat-spot scores, although in eight the cardiovascular autonomic score was still normal.

There was good correlation between loss of sweat glands and severity of COPD, particularly when this was judged by means of arterial oxygen tension (Pa_{O_2}) (fig. 2), or forced expiratory volume in one second (FEV_1) (fig. 3). The COPD patients also had evidence of a subclinical parasympathetic neuropathy, with preservation of the control of systolic blood pressure during postural change.

There were no significant correlations between age, arterial carbon dioxide tension (Pa_{CO_2}) and any other parameter (table 3). The sweat-spot score appeared to be a more sensitive predictor of subclinical autonomic dysfunction as assessed by Valsalva ratio (fig. 4) or the overall autonomic score (fig. 5).

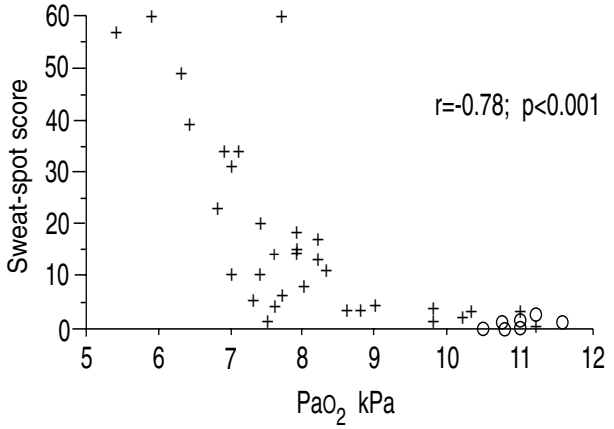

Fig. 2. – Acetylcholine sweat-spot scores and arterial oxygen tension in 35 hypoxic COPD patients (+) and 7 controls (○). Sweat-spot scores >4 are abnormal. For abbreviations see legend to figure 1.

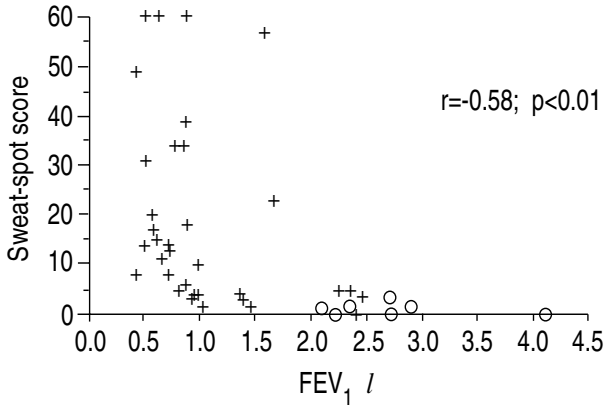

Fig. 3. – Acetylcholine sweat-spot score and FEV_1 in 35 COPD patients (+) and 7 controls (○). Sweat-spot scores >4 are abnormal. FEV_1: forced expiratory volume in one second; COPD: chronic obstructive pulmonary disease.

Table 3. – Spearman rank correlations (n=35 patients with COPD)

Parameters	r	p
Pao_2 *vs* ACh sweat-spot	-0.78	<0.001
Pao_2 *vs* Valsalva ratio	0.70	<0.001
Pao_2 *vs* 30:15 ratio	0.47	<0.01
Pao_2 *vs* autonomic score	0.61	<0.001
Pack-yrs *vs* Valsalva ratio	-0.46	<0.05
Pack-yrs *vs* sweat-spot score	-0.38	<0.05
Pack-yrs *vs* autonomic score	-0.47	<0.05
ACh sweat-spot score		
vs age	0.15	NS
vs FEV_1	-0.58	<0.001
vs $Paco_2$	0.35	NS
vs Valsalva ratio	-0.77	<0.001
vs breathing	-0.54	<0.01
vs 30: 15 ratio	-0.63	<0.001
vs postural BP fall	0.48	<0.01
vs autonomic score	0.80	<0.001

ACh: acetylcholine; NS: nonsignificant. For further abbreviations see legend to table 2.

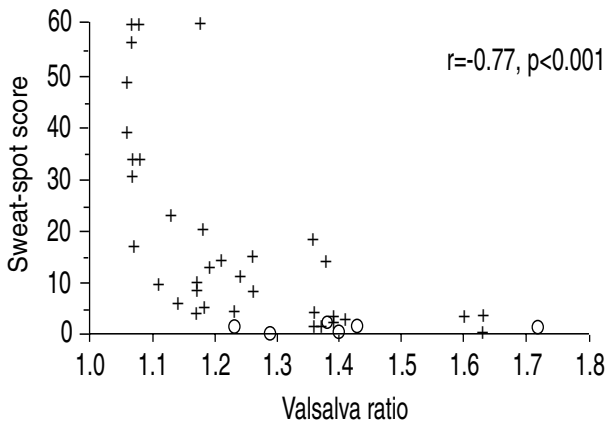

Fig. 4. – Acetylcholine sweat-spot compared with the Valsalva ratio in 35 COPD patients (+) and 7 controls (○). Sweat-spot scores >4 and Valsalva ratios <1.11 are abnormal. COPD: chronic obstructive pulmonary disease.

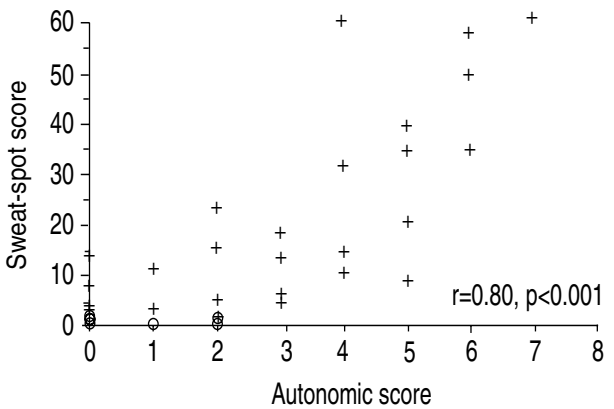

Fig. 5. – Acetylcholine sweat-spot scores compared to cardiovascular autonomic score in 35 COPD patients (+) and 7 controls (○). Sweat-spot scores >4 and autonomic scores >3 are abnormal. COPD: chronic obstructive pulmonary disease.

Discussion

The acetylcholine sweat-spot test results show that patients with COPD, particularly those with hypoxaemia, have evidence of a peripheral sympathetic autonomic neuropathy. The cardiovascular results confirm our previous findings that a subclinical parasympathetic autonomic neuropathy does occur in patients with COPD [5].

The acetylcholine sweat-spot test appeared to be a more sensitive marker for autonomic dysfunction. Eight of the less hypoxaemic COPD patients had an abnormal sweat-spot test, and yet normal (for their age) cardiovascular autonomic score. The converse did not occur. A longitudinal study is needed to confirm whether this abnormality in sweating is truly an early marker for future cardiovascular autonomic neuropathy. However, in general, the acetylcholine sweat-spot results correlated well with the commonly used diagnostic cardiovascular autonomic tests.

The correlation between sweat-spot result and Pao_2 was greater than that seen for the cardiovascular tests. This might reflect the avoidance of the many potential confounding factors inherent in tests which involve respiratory

manoeuvres and postural changes in generally infirm breathless subjects.

This high level of correlation between autonomic dysfunction and Pa_{O_2} suggests that hypoxaemia may be important in the development of autonomic neuropathy. Chronic hypoxia is a recognized cause of a peripheral neuropathy. APPENZELLER *et al*. [12] noted a symmetrical bilateral neuropathy and muscle wasting, whilst KINSMAN *et al*. [13] showed a high frequency of sensory disturbances in COPD patients with few clinical signs.

The peripheral neuropathy of hypoxic COPD and diabetes mellitus have pathophysiological similarities. Patients with COPD and those with diabetes mellitus show resistance to the normal phenomenom of ischaemic nerve conduction failure. In COPD the resistance correlates with the arterial oxygen tension at the time of testing [14].

Thickening of the endoneurial capillary basement membrane and an altered microcirculation is seen in both diabetes and COPD [4]. Such changes may account for the endoneurial hypoxaemia seen in diabetic peripheral neuropathy both in man [2], and in animal models [1]. Rats with streptozocin-induced diabetes developed a peripheral neuropathy, the biochemical and electrophysiological abnormalities of which could be partially reversed by supplemental 40% oxygen [15]. Likewise, rats reared in a hypoxic environment had electrophysiological abnormalities similar to those seen in experimental diabetic neuropathy [3]. The evidence suggests that endoneurial hypoxia may be an important early factor in the pathogenesis of peripheral neuropathy [1, 2]. We would suggest that a similar mechanism may be occurring in autonomic neuropathies.

The finding that patients with relatively normoxic COPD have normal autonomic function supports our view that the autonomic dysfunction seen is a consequence, rather than the cause, of COPD. However, it is possible that parasympathetic denervation of the heart, carotid body chemoreceptors and aortic baroreceptors might impair homeostatic regulation. Such changes could play a role in sodium and water imbalance and be a risk factor for arrhythmias; thus, influencing morbidity and mortality in COPD.

The sympathetic tests based on blood pressure responses are relatively crude, and only affected late in the disease process both in diabetes [17] and COPD. The acetylcholine sweat-spot test is much more sensitive, and suggests that the sympathetic nerves may be affected at the same time as the parasympathetic neurones. This result is similar to that found in diabetes mellitus [7].

The acetylcholine sweat-spot test has the added advantage of not being age-related [6, 7]. Cardiovascular autonomic function, on the other hand, does decline with age.

Cigarette smoking is a major aetiological factor in the development of COPD and COPD-related peripheral neuropathy [16]. It has several potential neurotoxic actions; carbon monoxide exacerbates tissue hypoxaemia, nicotine has stimulant actions and cyanogens may interfere with nerve function. However, the fact that the correlation between cigarettes smoked and autonomic dysfunction is much less clear cut than that between hypoxaemia and dysfunction raises questions as to its aetiological role in autonomic neuropathy.

It is highly unlikely that our findings are the consequence of the patients therapies. We have previously shown that ipratropium bromide and terbutaline have no effect on cardiovascular autonomic tests [5]. Although not formally tested in this study, it is unlikely that ipratropium bromide usage could account for our results. Many of the patients had never used the drug, the drug was not taken before the tests, and there was no association between results and prior usage both in terms of current or lifetime dosage.

The high incidence of subclinical autonomic neuropathy in COPD patients may have important implications with regard to prognosis. Diabetics [17] and alcoholics [18] with autonomic neuropathy have a high mortality. Many deaths are unexpected and sudden. They tend to occur under conditions of stress and hypoxaemia [19]. Inappropiate or defective autonomic responses to major events, such as septicaemia or hypoxaemia, can cause death. The presence of an autonomic neuropathy should be considered in all patients with hypoxaemic COPD, particularly in those with major illnesses or those undergoing anaesthesia.

In conclusion, the acetylcholine sweat-spot test is reproducible and easy to perform. Many patients with COPD show evidence of sweat gland sympathetic denervation. The results reaffirm the evidence that people with COPD have a subclinical autonomic neuropathy, which correlates with the severity of their hypoxaemia.

Acknowledgements: The authors wish to thank M. Dent who wrote the computer programme used to measure R-R intervals and R.E.J. Ryder whose assistance with the sweat-spot test was invaluable.

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