44 cases) than among patients with hepatitis B virus infection alone (15 out of 535 cases) ($p < 0.001$).

An interesting aspect of delta infection in chronic carriers is its connection with a depression in HBsAg titres. In the animal experiments by Rizzetto *et al*³ a lowering of HBsAg titres was seen in both carrier chimpanzees infected. The titres, however, eventually reverted to preinfection values. In our patients this effect is shown in fig 1, where it was temporary, and in fig 2, where the HBsAg titre stayed permanently below the sensitivity threshold of the assay and anti-HBs developed. This finding has diagnostic and prognostic implications. As the HBsAg titre decreases it may temporarily become difficult to detect the antigen. A correct diagnosis of an HBsAg carrier may then be missed. Previously, when less sensitive assays were used, such diagnostic mistakes were particularly liable to occur.

Delta infection in a chronic carrier is sometimes associated with termination of the carrier state. In our series of 191 HBsAg carriers, 19 lost HBsAg over 12 years' follow-up. Fourteen of these were drug addicts, among whom nine had and five had not demonstrable markers of delta infection. Thus, antigen clearance not infrequently occurs irrespective of delta infection. In three cases, however, clearance coincided with an attack of acute hepatitis and anti-HBs developed in all three cases, which suggests a causal relationship between delta infection and HBsAg clearance, a possibility which should be studied in more detail.

The discovery of the delta agent has improved our understanding of a number of obscure events in the natural history

of hepatitis B virus infection in drug addicts. Thus, acute episodes of hepatitis, sometimes called hepatitis non-A, non-B, may actually be instances of delta infection. The clearance of HBsAg not infrequently seen in drug-addict carriers may also be associated with delta infection.

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Raynaud's phenomenon and thermal entrainment: an objective test

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Abstract

A new objective test for diagnosing Raynaud's phenomenon was assessed in practice. The test is based on entrainment of the thermal vasomotor control system and entails non-invasive measurement of blood-flow responses in one hand while alternating thermal stimuli are applied to the contralateral hand. A significant ($p < 0.001$) abnormality of vasomotor control was found in patients with Raynaud's phenomenon compared with normal subjects.

When applied clinically this test is diagnostic and indicates the severity of the disease and the effect of treatment.

Introduction

Assessment and diagnosis of vasospastic disease of the digital vessels are often subjective, depending on the patient's descrip-

tion of the problem and the clinician's skill at interpreting physical signs that are often absent at the time of examination or difficult to elicit. Haemodynamic tests that may aid diagnosis include plethysmography,¹ Doppler studies of the patency of digital arteries,² and measurement of digital blood pressure.³ Unfortunately, the very nature of the intermittent and sometimes elusive vasospasm that occurs in Raynaud's phenomenon renders such tests liable to subjective and objective errors.

We describe a test for Raynaud's phenomenon that does not depend on the production of vasospasm and is free from observer error. The method is an application of thermal entrainment of the vasomotor control system and was developed as a result of investigation of this system in Raynaud's phenomenon.⁴

History

In 1940 Burton and Taylor⁵ found that peripheral blood flow is subject to spontaneous flow oscillations that occur at frequencies of less than 0.1 Hz. Kitney⁶ subsequently showed that by applying a certain range of periodic thermal stimuli to one hand the spontaneous variations in flow in the contralateral hand could be suppressed and replaced by strong oscillating variations occurring at the same frequency as the stimulus. This phenomenon is called entrainment and occurs when a stimulus is applied to an oscillating system at a frequency approaching its natural resonant frequency.

The ratio of the magnitude of the response of blood flow compared with the magnitude of the temperature stimulus is termed gain and can be precisely measured. It is the gain of the thermal vasomotor control system that is measured in thermal entrainment studies. The

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gain obtained is not solely dependent on an absolute measure of the increase or decrease in blood flow but is a measure of the variations in blood flow in response to the stimulus. It is not, therefore, subject to the usual errors incurred when measuring and comparing absolute volume flow rate.

Subjects and method

We investigated 26 subjects in a pilot study, enabling us to assess the method before introducing it into clinical practice. Of these, 10 were normal controls (four men and six women) and 16 were patients known to suffer from Raynaud's phenomenon (four men and 12 women). The mean age of the controls was 33 years (range 21-60) and of the patients 48 years (29-71). Subsequently we studied 27 patients referred consecutively for diagnosis of suspected Raynaud's phenomenon.

Subjects were seated in a quiet environment in a controlled ambient temperature of $22^{\circ} \pm 1^{\circ}\text{C}$. A thermistor was attached to the forefinger

FIG 1—Temperature stimulus to right hand and blood-flow response in left hand.

of one hand and a photoelectric plethysmograph to the distal phalanx of the forefinger of the contralateral hand.

After about 20 minutes the subjects had become acclimatised to the environment and the blood flow had stabilised. The hand with the thermistor attached was then immersed alternately in a hot (43°C) and cold (15°C) water bath at a steady rate. The rate of alternation was signalled to the subject by a system of flashing lights together with a low-intensity audible alarm. In this way the application of temperature stimuli at 0.0125, 0.0166, 0.025, 0.033, 0.05, and 0.1 Hz was precisely controlled. At each of these frequencies recordings were made of blood flow for 10 minutes (photoplethysmograph)⁷ and temperature (thermistor) on a frequency-modulation (FM) tape recorder and simultaneously fed on line to a computer for preanalysis. After each stimulus frequency a rest period of five minutes was allowed, during which preliminary online computer analysis was carried out to verify that the equipment was functioning correctly. Thus the result of the analysis was available to the investigator before the start of the next stimulus sequence. All measurements were carried out with the investigator out of the room, the subjects being monitored by closed circuit television. The blood-flow traces were analysed using a fast Fourier transform, which allowed the complex flow waveform obtained (fig 1) to be broken down into the range of the amplitudes of its constituent sine waves (fig 2). In this way the stimulus frequency that was producing the greatest variation of blood

flow could be determined, and hence the frequency at which the control system had the greatest gain.

Figure 2 shows a typical pattern of amplitude of flow at a stimulus frequency of 0.0125 Hz in a patient with Raynaud's phenomenon. The ratio of the area under the curve at the peak stimulus frequency to the area under the response curve is defined as the gain value. If the gain is then plotted against the stimulus frequency the characteristic in fig 3 is obtained.

FIG 2—Range of flow amplitudes and stimulus frequencies.

FIG 3—Gain ($\pm$ SD) obtained with each stimulus frequency.

Results

The highest gains in all subjects were found to occur at stimulus frequencies of 0.0125 Hz (40 seconds in hot water alternating with 40 seconds in cold water) and 0.025 Hz (20 seconds in hot, 20 seconds in cold). The results in both the control group and the group with Raynaud's phenomenon were found to be normally distributed about the mean values at each frequency. The patients with Raynaud's phenomenon had a significantly higher peak at 0.0125 Hz (Student *t* test, $p < 0.001$) than the controls, and this significance was maintained at 0.0166 Hz ($p < 0.001$). At higher frequencies the significant difference disappeared. Because of the highly significant difference between the low-frequency gains in the patients compared with the controls a graph was plotted of the gain at 0.0125 Hz against the gain at 0.0166 Hz for individual subjects (fig 4); the separation between the patients with Raynaud's phenomenon and controls may be clearly seen. One elderly woman with supposed Raynaud's phenomenon yielded results within the normal range, which we could not explain.

Having perfected this method of testing for Raynaud's phenomenon, we used it in 27 consecutive patients referred to our vascular laboratory for diagnosis. In this group measurements were made at only 0.0125 Hz and 0.0166 Hz; this reduced test was rapid (20 minutes), reproducible, and, when plotted in the manner shown in fig 4, of diagnostic value. The position of the plot for each patient appeared to be a measure of the severity of the disease, those patients in whom gross ischaemia and gangrene were present having the greatest gain values and those patients with only mild disease having gain values nearer normal. With this information the plot could be roughly divided into areas of severity according to the patient's history and clinical findings (fig 5). Because these 27 patients were not preselected in any way

before thermal entrainment testing several yielded results that fell within the normal range. Subsequent clinical examination of these patients using Doppler tests of the patency of digital arteries showed that only one patient, an 18-year-old girl, had demonstrable vasospasm of the digital arteries on cold provocation at 15°C. All the other patients had either functional problems or peripheral neuropathy.

FIG 4—Initial results, showing separation of patients with Raynaud's phenomenon from control subjects.

FIG 5—Results obtained in clinical practice (controls shown for reference).

Discussion

When the results obtained in normal subjects were assessed it appeared that two systems were being entrained, thereby producing the two increased gain values at stimulus frequencies of 0.025 Hz and 0.0125 Hz. These frequencies represented alternate hot and cold stimuli each lasting for 20 s and 40 s respectively.

In patients with Raynaud's phenomenon we found significantly increased gain values at 0.0125 Hz compared with normal controls, but not at 0.025 Hz or higher frequencies. This indicates that we were not simply measuring the reduced blood flow that occurs in Raynaud's phenomenon since if this had been the case we would have expected a significant difference across the whole range of frequencies. Furthermore, the results showed that there was a gross difference in thermoregulatory control in these patients at the lower frequency, but it is difficult to speculate on what caused this change and why the effects were only at low frequencies.

From known values of nerve conduction times,⁸ an afferent impulse from peripheral thermal receptors, causing a sympathetic

effect via the temperature centre in the hypothalamus, should produce a measurable response within seconds.⁹ Entrainment of such a system would give increased gain values at frequencies around 1 Hz. Our results cannot therefore be explained solely in terms of entrainment of the sympathetic nervous system, since the time constants are too long. At present it is impossible to impart thermal stimuli at about 1 Hz due to the experimental technique used, uncertainty of heat transfer at such high frequencies, and changes in blood flow caused by movement.

Since we were measuring blood-flow responses in the hand contralateral to the one receiving the thermal stimulus, it might be argued that the 40-second periodicity represented the circulation and reaction time of a naturally occurring humoral modulator of thermoregulatory control that is produced in response to thermal stimuli and is altered in Raynaud's phenomenon. This hypothesis is supported by a computer model of the thermal vasomotor control system.¹⁰

Several patients referred for assessment had previously undergone unilateral or bilateral sympathectomy. We found that these patients still exhibited a positive response to thermal entrainment, the gain value falling within the area expected from clinical history and findings. We tested only two patients before and after cervical sympathectomy. In both cases, however, the position of the gain value on the graph moved from the severe to the mild area in parallel with an improvement in the clinical condition, but neither entered the normal range.

The present study shows that an abnormality exists in the thermal vasomotor control system of patients with Raynaud's phenomenon. The abnormality appears to be largely unrelated to sympathetic innervation, is more pronounced in patients with severe disease, and may be accurately and objectively measured. Although unilateral tests of digital arterial patency are of great value in assessing Raynaud's phenomenon, they fail to take into account the bilateral aspect of the condition. We have incorporated thermal entrainment testing into our general assessment of Raynaud's phenomenon; it is proving to be a valuable diagnostic and research tool with which both the course of the disease and its treatment may be plotted.

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