Peripheral Venous Blood Concentrations of Epinephrine and Norepinephrine in Primary Raynaud's Disease

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Chromatographic separation and biologic assay of extracts from the peripheral venous blood of patients with primary Raynaud's disease have been performed. An increase in the venous concentration of epinephrine and norepinephrine was found, both under warm resting conditions and conditions of sympathetic stimulation by indirect cold, in patients with this disease.

The term Raynaud's disease, originally introduced following Raynaud's thesis in 1862 to classify cases of peripheral vascular disease which presented with pallor, cyanosis or gangrene of the extremities, is today generally accepted to comprise two main groups of patients. Whereas the diagnosis of idiopathic or primary Raynaud's disease is now only applied to those cases in which careful search has failed to reveal either a local precipitating cause or an associated systemic disease, the presence of one or other of these necessitates the use of the term secondary Raynaud's phenomenon.

In considering the etiology of the primary disease, considerable controversy exists concerning the relationship of the sympathetic nervous system to the vascular defect. Whereas Raynaud and later Allen and Brown considered that the abnormality consisted of a neurosis characterized by overactivity of the vasomotor nervous system, Lewis concluded that the disease was due to a local hypersensitivity of vessels of the order of the digital arteries to relatively low temperatures and the sympathetic nervous system was entirely normal.

In considering Lewis's conclusion it should be noted that in his original series several of the cases investigated, by virtue of associated disease, would no longer be generally accepted as constituting cases of the primary disease. Moreover, the observations on the role of the sympathetic nervous system were made in patients in whom trophic changes rendered it almost certain, as Lewis himself later established, that structural disease of the vessels was present.

The presence of subclinical degrees of organic arterial disease is extremely difficult to detect. Measurement of skin temperatures is unsatisfactory, as Cooper et al. have established that digital temperatures as high as 34°C can be reached when the cutaneous circulation is only partially dilated. Arteriography, although of value in isolating segmental blocks, has not yet reached the degree of technical development that it can detect minor changes of caliber in vessels of the order of the digital arteries; moreover the necessity for exact comparison with an adequate series of controls at all age groups, together with the almost invariable superimposition of vascular spasm due to the injection of contrast medium, renders interpretation difficult and potentially fallacious.

It can be shown by blood flow studies under conditions of vasodilatation that a considerable percentage of patients with uncomplicated Raynaud's disease fail to reach the degree of vasodilatation seen in normal controls. That this results from subclinical degrees of structural disease of the vessel wall must be regarded as highly probable, in view of the fact that individuals in whom the attacks of Raynaud's phenomenon are mild and not of such severity as to make them seek medical advice, react quite normally to procedures designed to induce vasodilatation of the hands and fingers. In many ways it would appear that the advent of organic
arterial obstruction is the determining factor in changing a mild case of Raynaud's disease to one which necessitates treatment for a disability.

In order to assess clinically the role of the sympathetic nervous system in Raynaud's disease, a necessary prerequisite must be that the cases analyzed should not only be clinically but also plethysmographically unobstructed, as the presence of disease in the vessel wall will almost certainly modify the reactions of the vessel to physiologic stimuli. It is not sufficient to assume because digits are free from trophic changes that the vessels themselves are not organically obstructed. Lewis and Allen have both drawn attention to the fact that in some such cases complete occlusion of the digital vessels can in fact be present without there being any clinical evidence that organic changes have occurred.

In view of the discovery by Von Euler that norepinephrine as well as epinephrine is present in sympathetic nerves and the demonstration by Peart and later Mann and West that norepinephrine is released in high concentrations when adrenergic nerves are stimulated, it was thought possible that analysis of the concentration of vasoconstrictor substances in peripheral venous blood might be of value in detecting whether an abnormality of the sympathetic nervous system is present in Raynaud's disease and it is with this aspect of the disease that this paper is mainly concerned.

**Methods**

**Clinical Material.** Eleven patients, aged 22 to 54 years, were selected from a large series available at University Hospital, Ann Arbor. They all fulfilled the criteria laid down by Allen and Brown as being cases of primary Raynaud's disease. They were chosen as being typical of a number of groups seen in clinical practice. Nine were female and two male. Cases 1 and 2 were clinically mild, the symptoms being nonprogressive and painless. Cases 3 to 10 were of moderate severity, pain being a dominant symptom, progression of the disease was occurring, but no secondary changes of pulp atrophy or ulceration had taken place. These were of interest as they form the main group of patients so often referred for surgical sympathectomy. Case 11 was regarded as severe, as in addition to pain and progression of symptoms, there was secondary ulceration of several digits.

Four of the cases had had previous upper dorsal sympathectomies and in all cases clinical relapse had taken place. The presence of sympahtetic nervous activity was confirmed in all 4 both by peripheral nerve blocks and indirect heating, the tests being carried out in conjunction with a thermostatically-controlled water-filled plethysmograph with the hand immersed at a temperature of 32°C.

All cases complained of the typical color triad of pallor, cyanosis and redness of the digits of the upper extremities, although the duration and intensity of the various stages varied from individual to individual. Moreover, a change in the characteristics of the color changes had occurred in some with the passage of time; the cyanotic stage, as is often the case, becoming more dominant and the stages of pallor and recovery less marked.

In 10 out of the 11 cases the symptoms were quadrilateral, although the lower limb manifestations were not as severe as those in the upper limbs.

The age of onset of symptoms ranged from 17 to 42 years and in cases 5 and 6 appeared to be definitely related to the onset of an operatively induced artificial menopause. In case 4 a definite exacerbation of symptoms followed the birth of her second child. Case 5 in addition had developed mild schizophrenia following the artificial menopause and was, at the time of the investigation, emotionally unstable.

In the 2 male patients, symptoms had been present for 20 years and 5 years respectively with no evidence of phlebitis or obliterative arterial disease.

**Control Series.** Six healthy medical students, 4 female and 2 male, ages 20 to 23 years, were investigated in the same way as the group of patients. The only selection in this group was that none of these individuals had ever suffered from pallor, cyanosis or chilblains of the extremities and they were in completely normal health at the time of investigation. The absence of demonstrable color changes was confirmed in each case by exposure to an environmental temperature of 14°C, under resting conditions for 30 min. with immersion of the hands in water at 18°C.

**Method of Investigation.** All patients were investigated under inpatient resting conditions and were taken to the laboratory in a wheel chair. The patient lay on a couch in a temperature-controlled room at 26 ± 1°C, wearing night attire.
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One hand was placed in a thermostatically controlled water filled plethysmograph at 32 ± 0.5 C. An indwelling venous trocar and cannula was inserted into a vein on the dorsum of the opposite hand or wrist, having previously been dipped in heparin to prevent intravascular thrombosis. The patient was then completely covered with blankets with the exception of the mouth, the nose and an 8" X 6" segment of the abdominal wall. This was left exposed and a metal duct of the same dimensions approximated to the exposed area. The duct was connected to an air conditioning plant and by means of a spring lever a blast of cold air at 4 C. and approximately 20 m.p.h. could be used as a controllable constant and repeatable indirect cold stimulus, the rest of the patient being completely screened from its effects. After this preparation the patient was left undisturbed for 30 min.

Six hand blood flow recordings were then made at 30 sec. intervals and a mean value calculated. Twenty to 50 ml. of blood was withdrawn from the indwelling venous cannula into a heparinized syringe. Ten minutes later a blast of cold air was directed onto the exposed abdominal wall for 3 to 4 min. and continuous blood flows recorded during the cold stimulation. At the same time a further blood sample was taken from the opposite hand.

The first blood sample taken at an environmental temperature of 26 ± 1 C. was labeled "warm." The second sample obtained during and immediately after the severe indirect cold stimulus was marked "cold."

The control groups were investigated in the same way under identical conditions.

Method of Analysis. The heparinized blood samples were immediately cooled, measured and centrifuged over ice. The plasma was removed by a pipette and measured. Ten volumes of acid ethanol were then added and the resultant mixture was thoroughly shaken and placed in a deep freeze at -23 C. for 12 hours to obtain complete protein precipitation. The mixture was then filtered and the filtrate distilled at 55 C. in vacuo to 5 to 10 ml. To this an equal volume of ether was added and the inorganic salts precipitated by the addition of 5 volumes of ethanol. The clear fluid was then distilled in vacuo at 55 C. to almost dryness and the residue taken up in 0.75-1 ml. of acid ethanol.

This was applied to an ascorbic acid-treated paper cylinder and chromatogrammed using water-saturated phenol as the solvent in an atmosphere of sulphur dioxide. The chromatography, elution and preparation of the elutes for biologic assay were performed according to the method described by Crawford and Outschoorn using 170 to 250 Gm. rats. A chromatogram blank was assayed in an attempt to detect vasoconstrictor activity.

RESULTS

The hand blood flows and the levels of vasoconstrictor substances are recorded in tables 1 and 2 (see also figures 1-3).

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by Crawford and Outschoorn using 170 to 250 Gm. rats. A chromatogram blank was assayed in each case. Standard solutions of synthetic amines were used to calibrate the blood pressure response of the animal and the injection samples were 0.025 to 0.1 ml., except in the case of the control series where samples up to 0.3 ml. were injected in an attempt to detect vasoconstrictor activity.

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DISCUSSION

The hand blood flows of the Raynaud's group of patients were considerably lower under both warm resting conditions and conditions of indirect cold stimulation than the hand blood flows of normal controls. Under both conditions there was evidence that an abnormal degree of peripheral vasoconstriction was present in the cases of peripheral vascular disease. The hand blood flows of similar groups compared under resting conditions at an environmental temperature of 20 \( \pm \) 0.5 C. and a plethysmograph temperature of 32 C. showed similar differences. In the Raynaud's group a mean value of 2.9 ml./100 ml./min. (S.D. = .97) was obtained in 28 cases compared with 6.3 ml./100 ml./min. (S.D. = 1.65) in 20 normal controls.\(^{14}\) It is of interest that the hand blood flows of 2 cases of pheochromocytoma measured under the latter conditions were 3.8 and 4.0 ml./100 ml./min. during periods when the systemic blood pressure was elevated to 300/210 and 240/130 mm. Hg respectively. This would imply that a greater degree of peripheral vasoconstriction is present in the hands of cases of Raynaud's disease in whom a normal blood pressure is the rule than in the condition of generalized systemic hypertension induced by a pheochromocytoma.

The values of epinephrine and norepinephrine in the peripheral venous blood at the wrist in the cases of Raynaud's disease were high and it is of interest that the norepinephrine values during cold stimulation appeared to be related to the clinical severity of the disease, being lowest in the mild cases (cases 1 and 2) and highest in the most severe case in the series (case 11). In analyzing the reason for these high values it is obviously necessary to relate the normal values for human blood to the exact site of sampling and conditions under which the samples are taken. It is believed that the substances measured originated in the main if not entirely from the sympathetic nerve...
endings, as following a complete sympathectomy, even during the immediate postoperative phase when hypersensitivity to circulating vasoconstrictor substances is maximal, the degree of peripheral vasoconstriction that can be induced by indirect cold is relatively small and of little practical importance. Moreover, Von Euler\textsuperscript{15} has shown that although the urinary excretion of epinephrine decreases considerably following bilateral adrenalectomy, it does not fall to zero and the norepinephrine fraction is either unchanged or increased. The values obtained, therefore, particularly for norepinephrine, must not only be related to the degree of sympathetic tone present at the time of sampling, but will vary according to whether it is taken from a superficial vein draining an area where sympathetic activity is high or one draining an area where this is low or absent.

In considering other factors that could influence peripheral venous levels, Smith\textsuperscript{16} has demonstrated that both the degree and duration of epinephrine induced vasoconstriction are considerably influenced by lowering the temperature of the arterial wall and concluded that this was due to the effect of temperature on the enzyme systems which inactivate the constricting agent. In Raynaud's disease the average digital cutaneous temperature in a room temperature of 20 ± 0.5°C is 22.3°C compared with 30.2°C in normal individuals;\textsuperscript{14} similar differences have been reported at an environmental temperature as high as 25°C.\textsuperscript{17} Over this range of temperatures, therefore, the intraluminal temperature of the blood in the digital arteries of patients with Raynaud's disease is probably considerably lower than that seen in normal controls, due to the precoothing of arterial blood.\textsuperscript{18} In this connection, the amine oxidase content of 2 digital arteries taken from a finger amputated for terminal gangrene in a case of Raynaud's disease in a 22-year-old female is noteworthy. The proximal portions of the arteries were patent, microscopically normal and no amine oxidase could be detected. In two other digital arteries taken from 2 digits amputated for nonvascular reasons, the amine oxidase activity was 552 μl O₂/Gm./hr.\textsuperscript{*} It is possible therefore that the intense peripheral vasoconstriction and high concentration of vasoconstrictor substances seen in these patients could be related to the incomplete destruction of the amines liberated from the peripheral sympathetic nerve endings in the walls of the arteries of the hand and digits. Von Euler,\textsuperscript{19} following reflex vasomotor stimulation of normal subjects by tilting, has found considerable increases in the urinary excretion of epinephrine and norepinephrine, the increase being in some cases as much as 800 per cent. He has suggested as the result of experiments in adrenalectomized subjects\textsuperscript{20} that physiologic activity can give rise to an overflow of norepinephrine into the circulating blood. This was not detected with reflex cold stimulation, using the methods described in the normal control series, but was apparent in the cases of primary Raynaud's

\*These were performed by Professor R. H. Thompson, Guy's Hospital, London, whose help is gratefully acknowledged. The estimations were determined manometrically using Warburg technic and tryptamine substrate.\textsuperscript{25}
disease. Moreover the degree of peripheral vasoconstriction in Raynaud's disease is far greater than can be induced in normal controls by either tilting or reflex cold stimulation, these procedures never being accompanied by any evidence of peripheral vascular insufficiency.

In considering the systemic blood levels one would not expect venous concentrations of this order to have any effect on the general blood pressure because of their slow rate of entry into the general circulation. The total blood flow from both hands under the warm resting conditions detailed would seldom exceed 40 to 50 ml./min.; under cold stimulation the figure would be very considerably reduced to a maximum of 10 to 15 ml./min. With such rates of flow only relatively small amounts of amines would enter the systemic circulation per minute and would not be sufficient in themselves to influence the systemic blood pressure levels.21

**Summary**

The concentrations of epinephrine and norepinephrine in the peripheral venous blood at the wrist of 10 out of 11 patients with primary Raynaud's disease were found to be higher than normal when estimated by chromatography and biologic assay under warm resting conditions. An increase, mainly of the norepinephrine fraction, which appeared to reflect the clinical severity of the condition was found in most cases following sympathetic nervous stimulation by cold.

The high concentrations are considered to be due to an abnormality of the metabolism of these substances which probably arise mainly, if not entirely, from the peripheral sympathetic nerve endings. The possible reasons for this abnormality are discussed and a report is made on the amine oxidase content of 2 digital arteries taken from the digit of a case of primary Raynaud's disease.

**SUMMARIO IN INTERLINGUA**

Le concentrationes de epinephrina e de norepinephrina in le sanguine periphero-venose del carpo de 10 ex 11 patientes con primari morbo de Raynaud se monstrava plus alte que normal quando estimate per chromatographia e essayo biologic sub conditiones calido e de reposo. Un augmento, principalmente del fraction de norepinephrina e reflectente apparentemente le severitate clinic del condition, essava constatate in le majoritate del casos post stimulation del sistema nervose sympathic per exposition a frigido.

Es opinate que le alte concentrationes observate es le effecto de un anormalitate in le metabolismo del substantias in question e que iste substantias se deriva primarimente —si non completely—ab le terminations del peripherie nervos sympathetic. Le rationes possibile de iste anormalitate es discu-tite. Es presentate un reporto relative al contento de amino-oxydase in duo arterias digital obtenite ab un paciente con primari morbo de Raynaud.

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