

the endotoxin is dissolved in whole blood. This is probably due to uptake of the endotoxin by platelets (Herring *et al.*, 1963; Das *et al.*, 1973).

The limulus test appears to fulfill most of the criteria for a simple test for detecting endotoxin *in vivo*. Recent reports on the value of the test in clinical practice have varied. Levin *et al.* (1970, 1972) are of the opinion that the test is useful but that endotoxaemia and bacteraemia may not occur concurrently. Stumacher *et al.* (1973) had reservations about its value as a test for the detection of Gram-negative infection. It is apparent that those who report a good correlation between a positive test result and the presence of Gram-negative infection have used the test in selected patients (Levin *et al.*, 1970, 1972; Das *et al.*, 1973), whereas Stumacher *et al.* (1973) studied a more general population but did not furnish any clinical information about their patients, which may have helped to establish a relation between the results of the limulus test and other pathological processes. Caridis *et al.* (1972) provided detailed clinical information on some patients and related this to the findings of the limulus test.

In our study 72% of the limulus tests performed on blood samples from patients with active local or systemic Gram negative infections gave positive results. After the patients had recovered clinically only one positive result was obtained. These figures indicate that Gram-negative infection is not always accompanied by a persistent endotoxaemia, and because of its transient nature the limulus test should be repeated if reliable results are to be obtained.

Endotoxaemia also occurs in the absence of Gram-negative infection, and in the six patients in this series with "chemical peritonitis" 76% of the limulus tests gave positive results, the figure falling to 18% after the signs of peritonitis had settled. It has been postulated that endotoxin may be released from abnormally permeable bowel and absorbed into the circulation via the peritoneal cavity. Fine *et al.* produced experimental evidence which provides the basis for this hypothesis (Tamakuma *et al.*, 1971; Cuevas and Fine, 1972) and our findings lend it clinical support.

In the 23 patients without overt Gram-negative infection or peritonitis 15% of the limulus tests gave positive results. Though precautions were taken to ensure that contamination of the glassware and lysate did not occur, by performing simultaneous control tests contamination of the specimen during collection cannot be entirely ruled out. The other possibility is that the

limulus lysate is sensitive to the presence of such small quantities of endotoxin; it is quite possible that these patients did have endotoxaemia but not in amounts sufficient to cause clinical symptoms or signs. This is almost certainly true of the patient with the reversed ileal loop, who had five positive test results before the loop was excised.

We feel that the limulus test is a simple, rapid, and reliable method for the detection of endotoxaemia, particularly when it is repeated frequently during the episode of infection. The clinical significance of small amounts of circulating endotoxins detected by the method has yet to be determined. It is possible that the tests may be useful for assessing the response of treatment to control Gram-negative infection and as a screening procedure for the presence of endotoxaemia in patients at high risk of developing endotoxic shock. Early and vigorous treatment at this stage may reduce the mortality and morbidity due to this condition. Furthermore, it may also prove useful in relating the pathophysiological changes which occur in patients to the presence of endotoxaemia. We are at present engaged in investigating these possibilities.

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Sequelae of an ¹²⁵I-Fibrinogen Detected Thrombus

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Summary

Forty-four patients who had a deep vein thrombosis detected with the fibrinogen uptake test were followed up three to four years later. The mild aching pains and ankle swelling that were present at the time of the thrombosis persisted or got worse in many, but there was no increase in the incidence of varicose veins. The minor self-limiting thrombosis detected with the fibrinogen uptake test should not be considered innocuous, for it can cause long-lasting symptoms.

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Introduction

The development of the fibrinogen uptake test into a practical clinical test by Atkins and Hawkins (1965), Flanc *et al.* (1968), and Negus *et al.* (1968) enable us to detect the many small thrombi that occur in the leg veins after surgery and medical illnesses. Studies in many centres showed that though small thrombi are common most do not propagate but gradually disappear. So common is this self-limiting calf vein thrombosis that those using the fibrinogen uptake test adopted a policy of treating only those thrombi that extended beyond the calf or began above the level of the knee joint, on the assumption that a small calf thrombus was unlikely to become a serious embolus or significantly disturb the functions of the peripheral veins. No one has published a late follow-up of the state of the limbs of patients who have had a positive fibrinogen uptake test result, however, and this study attempts to remedy this deficiency.

Methods

We used the technique described by Negus *et al.* (1968). During 1968-71 while using the ^{125}I -fibrinogen uptake test (F.U.T.) to diagnose deep vein thrombosis in clinical trials of methods of prophylaxis we found 98 patients with positive results out of about 350 studied, and it is these who were followed up.

A questionnaire was sent to all the patients. They were asked whether they had any symptoms in their legs, such as pain, swelling, varicose veins, skin changes, ulceration, etc., and if so whether these were new and when they had developed. They were also asked to attend a special clinic, where they were questioned on their answers to the questionnaire and fully examined to determine the presence of varicose veins, swelling of the legs, and changes in skin of the leg, particularly eczema, pigmentation, and ulceration. Care was taken to exclude other causes of leg swelling before accepting that the swelling was related to the thrombosis. The patency of the popliteal and femoral veins was tested with a Doppler ultrasound flow detector. The patients were also asked if they had suffered any dyspnoea, orthopnoea, or chest pain since their operation.

Results

Of the 98 patients 31 had died from various causes, none to our knowledge from pulmonary embolism. Twenty-three had moved house and could not be traced, leaving 44 patients (88 legs) to study.

Of the 44 patients 32 were men, and 12 were women. This preponderance of males reflected the fact that much of our clinical research was with men undergoing prostatectomy. The mean age of all 44 patients was 59.7 years. The mean age of the 32 men was 58.5 years and of the 12 women 62.9 years.

The mean period of follow-up was three and a half years. The range was from 25 to 60 months, but the follow-up was less than 36 months in only nine of the 44 patients. Thus 80% of the traceable patients were being studied more than three years after their thrombosis.

THE ORIGINAL THROMBOSIS

Sixty-one of the 88 legs reviewed had had a positive F.U.T. result (F.U.T. positive), 27 had been normal (F.U.T. negative). The thrombosis was unilateral in 27 patients, and bilateral in 17 patients. The thrombus in the 61 positive legs was confined to the calf in 54, the thigh in one, and at separate sites in the calf and thigh in six. In only three of the patients with bilateral thrombosis was the distribution asymmetrical. In one patient it was in the calf of one leg and in the thigh of the other, and in two it was in the calf on one side and in the calf and thigh on the other side. All the other bilateral thrombi were confined to the calves. No patients were treated with anticoagulants.

The signs and symptoms that were present at the time of the positive F.U.T. result are listed in table I. More than half the patients had no symptoms in the leg (excluding varicose veins) at the time the test gave a positive result. A third had mild pain and tenderness in the calf, and a sixth had slight

TABLE I—Initial Symptoms and Signs in 61 Thrombosed Legs

No. (%)	None	Pain	Swelling	Pain and Swelling	Varicose Veins	Varicose Veins with Pain and/or Swelling
	16 (25)	15 (24)	5 (8)	6 (9)	27 (44)	8 (13)

swelling of the ankle. Nearly half the legs (44%) had some venous varicosities, most being small, symptomless, and not noticed by the patient.

A fifth of the patients had symptoms in the chest, which could have been attributed to pulmonary embolism or pneumonia. No special tests such as lung scanning were performed to help clarify the diagnosis.

One of the 27 F.U.T.-negative legs was painful and tender, and 12 contained varicose veins.

CONDITION OF LEGS AT FOLLOW-UP EXAMINATION

Pain.—The pain which was present in 21 of the legs at the time of the thrombosis was still present in 10 but had completely disappeared in 11 (table II). The pains were aching in nature, in the calf, occasionally associated with muscle tenderness, and usually exacerbated by prolonged standing. The one leg which had given a negative F.U.T. result and which had been painful after operation was better at follow-up examination. New aching pains had developed in five limbs, of which three had been F.U.T. positive and two F.U.T. negative. Thus 21% of the F.U.T.-positive legs and 8% of the F.U.T.-negative legs were painful at follow-up, compared with 34% and 4% initially.

TABLE II—Incidence of Aches and Pains, Swelling of Lower Leg and Ankle, and Varicose Veins in 44 Patients who had Thrombosis. Results expressed as Number (%) of Legs

	Before Thrombosis	At Time of Thrombosis	3½ Years Later
<i>Proportion of Legs with Aches and Pains</i>			
Positive F.U.T. result (61 legs)	0	21 (34)	13 (21)
Negative F.U.T. result (27 legs)	1 (4)	1 (4)	2 (8)
<i>Proportion of Legs with Swelling of Lower Leg and Ankle</i>			
Positive F.U.T. result (61 legs)	0	11 (18)	14 (23)
Negative F.U.T. result (27 legs)	1 (4)	1 (4)	3 (11)
<i>Proportion of Legs with Varicose Veins</i>			
Positive F.U.T. result (61 legs)	27 (44)		35 (57)
Negative F.U.T. result (27 legs)	12 (44)		15 (55)

Swelling.—Swelling of the ankle was still present in eight of the 11 legs that were swollen when the F.U.T. result was positive, but had disappeared in the other three (see table II). The swelling that had been present in one F.U.T.-negative leg was better. New swelling had appeared in nine legs, of which six had been F.U.T. positive and three F.U.T. negative. In none of these nine legs was there any obvious cause for the swelling, local, cardiac, or renal. Thus 23% of the F.U.T.-positive and 11% of the F.U.T.-negative legs were swollen at follow-up, compared with 18% and 4% initially.

Varicose Veins.—Varicose veins had appeared in 11 of the 49 legs that had been free from them at the time of thrombosis. Eight of these legs had been F.U.T. positive and three F.U.T. negative. Thus at the time of the follow-up examination 57% of the positive limbs had varicose veins, compared with 44% at the time of thrombosis, while the incidence of

TABLE III—Types of New Varicose Veins in Normal Legs at Time of Thrombosis. Results expressed as Number (%) of Legs

	Visible Varicosities Only	Varicosities + "Ankle Flare"	Preulceration Changes in Skin	Total
Positive F.U.T. result (34 legs)	2 (6)	4 (12)	2 (6)	8 (24)
Negative F.U.T. result (15 legs)	1 (7)	1 (7)	1 (7)	3 (20)

varicose veins in the 27 normal legs had risen from 44% to 55% (see table II). The type of new varicose veins that developed is shown in table III. If the ankle flare is accepted as a sign of perforator vein incompetence then six of the eight F.U.T.-positive and previously normal legs which developed new varicose veins had evidence of perforator vein incompetence, compared with two of the three legs in the negative result group, an insignificant difference. None of the varicose veins that were present at the time of the thrombosis got significantly worse.

Doppler Ultrasound Examination.—In none of the patients was there any clinical evidence of further thrombosis of the major veins above the knee. Of the 88 legs 56 were examined with the Doppler flow detector and all had normal flow patterns.

Pulmonary Embolism.—No patients had had any symptoms suggestive of pulmonary embolism between their discharge from hospital and the follow-up examination.

Discussion

Our general impression after reviewing these patients was that there was no disabling late morbidity that could be attributed to the thrombosis. We were surprised, however, that many of the minor symptoms had persisted for so long. The initial incidence of aches and pains in the limbs in which the F.U.T. gave positive results was 34%, compared to 4% in the normal limbs. As pain was not present before the thrombosis, with the exception of the pain in the one F.U.T.-negative leg, it is reasonable to attribute it to the thrombosis. The follow-up showed that one-half of the patients who had pain at the time of their thrombosis still had some discomfort three years later, and a few painless legs had become painful. Though this was a mild symptom some of our patients found it extremely annoying.

Swelling of the ankle also persisted in three quarters of the legs that were swollen at the time of the thrombosis. New swelling appeared in equal proportions in the thrombosed and normal legs. As it is difficult to exclude all other causes of swelling it would be wrong to attribute all cases of ankle swelling

to the thrombosis. Nevertheless, there was little doubt that the swelling was due to the thrombosis in some of the younger patients and that it persisted for three years.

The high incidence of varicose veins in our patients was probably due to the fact that they were an elderly group and we classified any tortuous, subcutaneous dilated vein as a varicose vein. The incidence of new varicose veins was the same in the legs which had been thrombosed as the legs that had been normal so nothing in our data suggests that the small F.U.T.-detected thrombus damages the perforating veins and predisposes to the postphlebotic leg. Interestingly, most of the new veins that developed in both the F.U.T.-negative and the F.U.T.-positive legs were of the type associated with perforator vein incompetence. As neither group had any episodes between the time of the thrombosis and the follow up examination suggestive of further thrombosis the cause of these new veins remains unexplained.

We conclude that though the minor form of deep vein thrombosis, which is a common complication of all operations and illnesses, is not a serious condition it can cause long-lasting symptoms. If the calf aches at the time of the thrombosis it is likely that it will continue to ache for at least three years. This is a minor symptom, and the patients that we interviewed had come to accept it. Nevertheless, the fact that it did persist suggests that the fibrinogen detectable thrombus is not an entirely benign condition and may disturb the physiology of the peripheral veins. We have no evidence to suggest, however, that the physiological disturbance is sufficient to cause varicose veins. We do not think that the persisting symptoms we have described are severe enough to justify treating the initial thrombosis with anticoagulants because such treatment is probably far more dangerous than the symptoms, but it may be worthwhile preventing these thrombi with a safe form of prophylaxis.

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Interaction between Doxycycline and some Antiepileptic Drugs

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Summary

The mean half life of doxycycline given to seven patients on long-term diphenylhydantoin treatment was 7.2 ± 0.4 hours. In five patients on long-term carbamazepine treatment the

half life was 8.4 ± 1.4 hours. In four patients on combined diphenylhydantoin and carbamazepine treatment the half life was 7.4 ± 0.7 hours. All these were significantly shorter than a mean half life of 15.1 ± 1.0 hours when doxycycline was given to nine control patients. Therefore doxycycline in normal doses given to patients taking diphenylhydantoin or carbamazepine may fail to maintain the minimum inhibitory concentration necessary for proper bacteriostasis. When doxycycline is given in association with agents known to induce drug metabolism the serum concentration of the antibiotic should be watched to see that bacteriostatic levels are maintained.

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Introduction

Treatment with a bacteriostatic antibiotic is ineffectual unless its serum concentration is continuously maintained at or above the minimum inhibitory level. Doxycycline seems to