Acute and Recurrent Thromboembolic Disease:
A New Concept of Etiology

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ACUTE thrombophlebitis with pulmonary embolism continues to be a common disease, and there has been little or no evidence of any decrease in its incidence in the last 25 years.[2] One of the chief problems in finding a method for decreasing its incidence or developing a more successful plan of treatment has been the inability to establish a specific etiology.

In the past, attempts have been made to establish an etiologic relationship between infection and thromboembolic disease. Studies by a number of investigators had occasionally demonstrated the association of various types of severe infection with acute thrombophlebitis.[1, 4, 15, 16] Most of these were instances of sepsis with high fever and septic embolism caused by the *Staphylococcus aureus,* the aerobic or anaerobic Streptococcus, or the *Bacteroides.* In such instances, the recognizable parent forms of the bacteria were isolated from the blood and identified.

De Takats in 1932[5] pointed out that "resting" or dormant infection in varicose veins was probably an etiologic factor in thrombophlebitis occurring after injection treatments, operations, trauma, or exposure to x-ray therapy. Bacteriologic studies of varicose veins at the time of ligation were made by De Takats and Kendall and over one half were positive. The resting bacteria found in this type of latent infection were studied by Kendall *et al.*[12] but the possibility of "L" forms or "filterable" forms was not suggested.

During the past 6½ years, the possibility that an obscure infection by an unusual organism, of atypical morphology and difficult to grow and recognize, might be the cause of many cases of acute or recurrent thromboembolic disease has been investigated in the Surgical Research Laboratory of the University of Cincinnati. In our original studies, "L" forms were found in the cultures of blood in surgically removed thrombi in 31 patients with thromboembolic disease, using special bacteriologic methods. The results of this initial study were reported at the Clinical Congress of the American College of Surgeons.[2]

The evidence thus accumulated suggested the possibility that bacteremia by "L" forms and other atypical bacterial forms may play a significant role in the etiology of acute or recurrent thromboembolic disease. Furthermore, a relationship of sex to two age periods of peak incidence was of particular interest. In the

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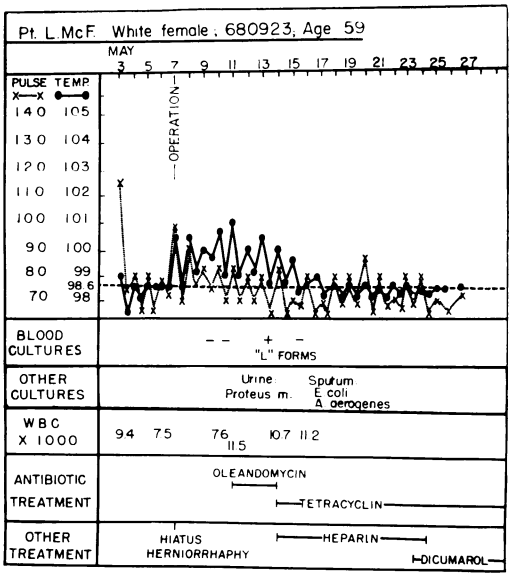

FIG. 1. Chart illustrating the findings and clinical course of a Type I patient who developed a deep thrombophlebitis of the right leg, a single pulmonary embolus on the seventh postoperative day after repair of a diaphragmatic hernia, and a transient "L" form bacteremia.

first age period of 20 to 39 years, 13 of 15 patients were women, one being postpartum, one having an infected abortion, and nine receiving Enovid therapy for contraceptive or therapeutic purposes. In the second age period of 45 to 75 years, nine of 13 were men, and five of these had a proven malignant tumor.

These data made us suspect that a hormonal relationship existed either in stimulating the spontaneous growth of the "L" forms or in favoring the development of one or more mechanisms which increased the coagulability of blood or disturbed the normal clotting mechanism.

Recent experiences have yielded an additional 19 patients with acute or recurrent thromboembolic disease and bacteremia with "L" forms and other atypical bacterial forms, making the total number investigated 50. Our studies of these cases have confirmed our previous data and have emphasized the possibility of a causal relationship between this type of bacteremia and the thromboembolic disease.

Material and Methods

Both clinical and laboratory studies of patients with spontaneous or recurrent thromboembolic disease have been made in the Department of Surgery and the Surgical Research Bacteriology Laboratory of the University of Cincinnati during the past 6½ years. The clinical studies were based upon the data obtained in the observations and treatment of 50 patients who were demonstrated to have "L" form or atypical bacteremia during their attacks of thromboembolism. Attention was paid to the relationship of various predisposing and contributing etiologic factors, and from this has been evolved a clinical syndrome with significant characteristics useful in its recognition and diagnosis.

The laboratory studies were concerned with bacteriologic procedures and technics necessary for the successful growth, identification, and study of these "L" forms and other atypical bacterial forms.

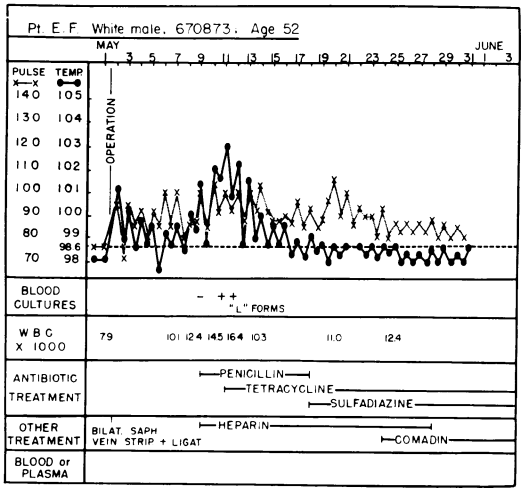

FIG. 2. Type II patient with recurrent thromboembolic disease who had three previous attacks of thrombophlebitis in the past 5 years. Large doses of heparin were required until 72 hours after start of tetracycline therapy.

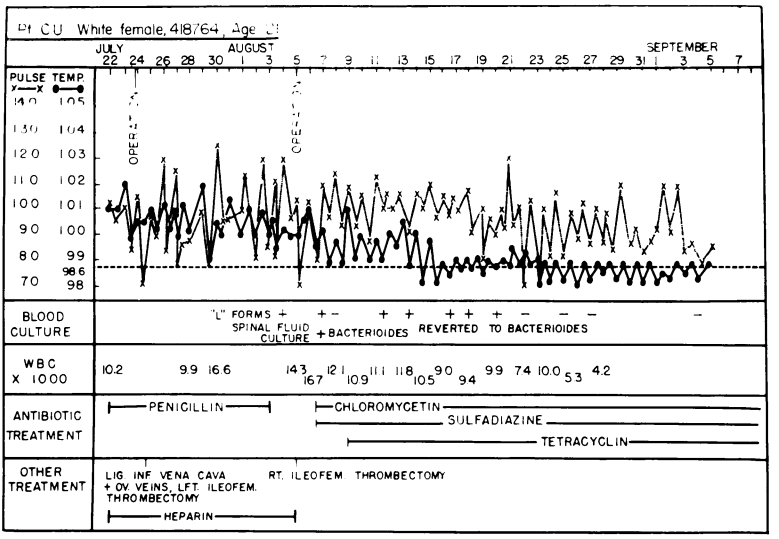

FIG. 3. Clinical Type II patient with recurrent episodes of acute thrombophlebitis, pulmonary emboli, and a persistent bacteremia with "L" forms which reverted back to parent *Bacteroides*. Growth of "L" forms stimulated by norethynodrel and *in vitro* interference with chemical assay and activity of heparin.

Clinical Studies

Clinical Types. Three general types were observed. The first consisted of 18 patients with acute thrombophlebitis and one attack of pulmonary embolism (Fig. 1). The second type was more common and consisted of 28 patients with multiple or recurrent episodes of thrombophlebitis with pulmonary embolism (Fig. 2). In the latter group, 17 patients continued to have one or more attacks of venous thrombosis and pulmonary embolism after vena caval ligation or thrombectomy (Fig. 3). In three of these instances the thromboembolic disease recurred over a 21- to 30-year period, and in one instance led to 22 periods of hospitalization. The third group included four patients who developed thromboembolic disease of the systemic venous and arterial circulations (Fig. 4). The arterial emboli consisted of two of the saddle aorto-iliac type and two involving multiple arteries with infarctions.

Age. The ages varied from 18 to 74 years, the average being 37 for the women and 49 for the men. Twenty-four or approximately one half of the patients were between the ages of 20 and 42. The other period of increased incidence was between 55 and 75 years.

Sex. Twenty-six were women and 24 were men. In the 24 patients in the age group between 20 and 42, there was an

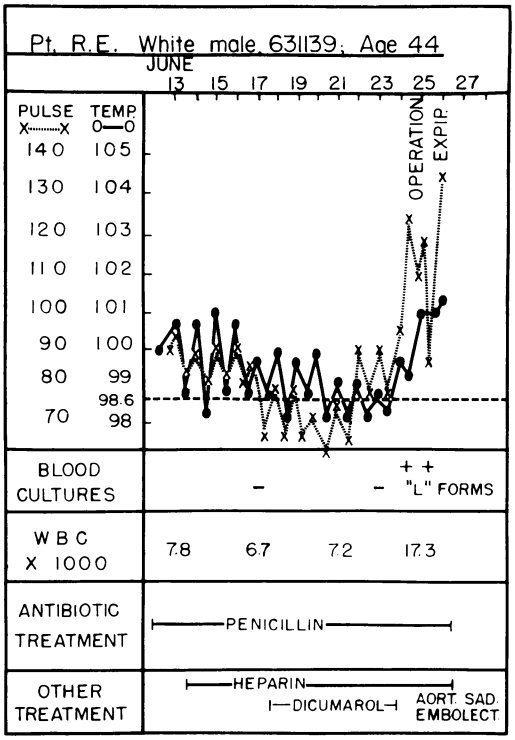

FIG. 4. Chart of Type III patient with multiple recurrent attacks of venous thrombosis, multiple pulmonary emboli, aortic saddle embolus, and "L" form bacteremia.

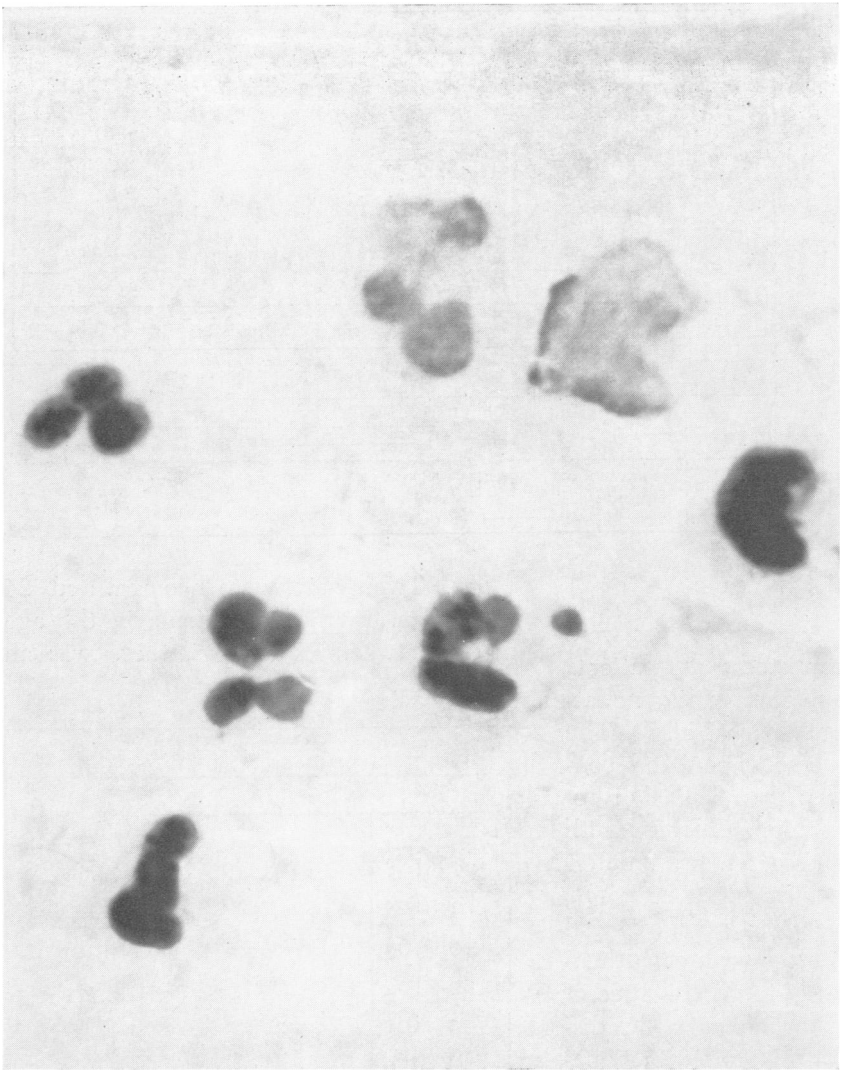

FIG. 5. Spherical bodies of a *Sphaerophorus sp.* resembling human erythrocytes with basophilic stippling. Colony on agar cut-out. Brightfield. Photographed at 1,188 ×. Phase-microscopy reveals numerous small granules.

interesting incidence of 20 women. Of these, four were immediately postpartum or postabortion, and 11 had been receiving Enovid for birth control.

Race. Thirty-five patients were Caucasians, and 15 were Negroes.

Trauma. In 35 patients the thromboembolic disease developed spontaneously without antecedent trauma or surgical operation. A preceding operation was noted in 13 patients and a serious injury in two.

Malignant Neoplasm. A proven malignant tumor was present in only seven of the 50 patients, being carcinoma of the pancreas in two, carcinoma of the lung in one, carcinoma of the prostate in two, carcinoma of the ovary in one, and lymphoma in one.

Clinical Evidence of Infection. There were variable clinical signs of infection evident in these patients. In some the fever, tachycardia, and leucocytosis were minimal; in others they were moderate or marked in degree. The lowest temperature at the time the "L" forms were recovered from the blood was 98° F. and the highest

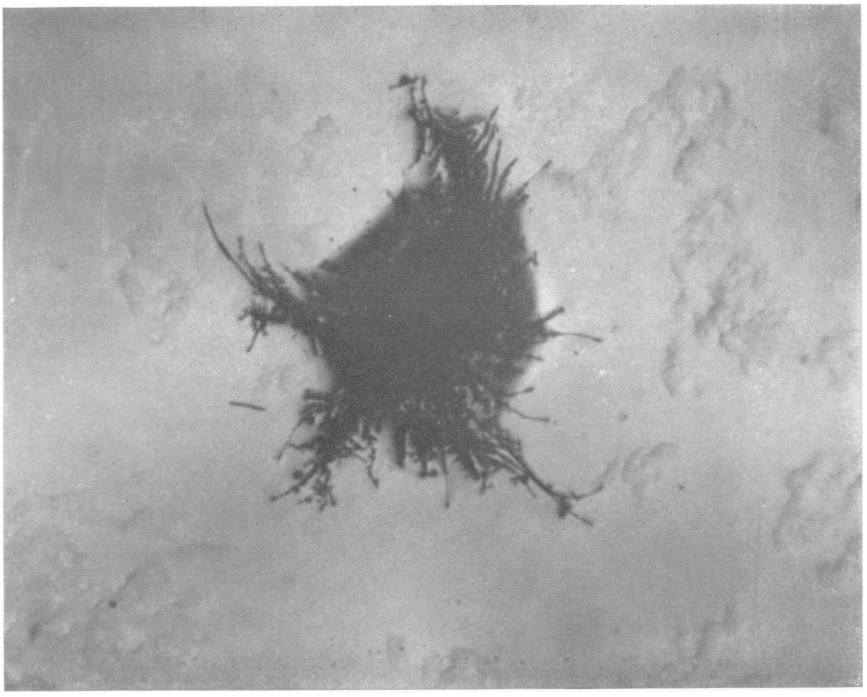

FIG. 6. "L-type" colony of a *Sphaerophorus sp.* grown on agar containing 2,3,5-triphenyltetrazolium chloride. Note the "tendrils" which grew into as well as along the surface of the agar (Photographed at 156×).

was 103.8°, the average being 100.8°. The pulse varied between 84 and 130, the average being 108. The white blood count varied between 4,500 and 17,900 with an average of 11,800.

Obvious sources of the "L" form infection were usually absent or obscure. A recognizable primary focus was demonstrated in seven patients, these being chronic active mastoiditis in one, parametritis in two, chronic tubo-ovarian abscess in three, and urinary tract infection in one. Clinical and laboratory evidence of encephalomeningitis was documented in five patients during the course of their thromboembolic illness.

Heparin Requirements for Anticoagulation. During the course of treatment of these patients with intravenously administered heparin, relatively high doses of 12,000 to 14,000 units every 4 to 6 hours were required in some instances to maintain a coagulation time of 25 to 35 minutes. After treatment for 48 to 96 hours with appropriate antibacterial therapy, the dosage of heparin required to maintain similar coagulation time was often markedly reduced along with the decreases in temperature, pulse, and white blood counts.

Bacteriological Studies

Specimens of blood, thrombi, and spinal fluid were cultured as available in Castaneda blood culture medium primarily,[3] and in Brewer Modified Thioglycollate Medium (BBL), with and without the addition of 0.6M sucrose. Subcultures were planted on a battery of media described earlier.[7, 11] All agar plates were incubated aerobically and anaerobically by use of the Mueller, Miller modification of the Rosenthal chromium-sulfuric acid method[14] or by use of the Gaspak jar with CO_2 (BBL) for a minimum of 7 days.

Primary and subcultures were examined by Gram and Giemsa stained smears. In addition, wet-mounts of broth cultures

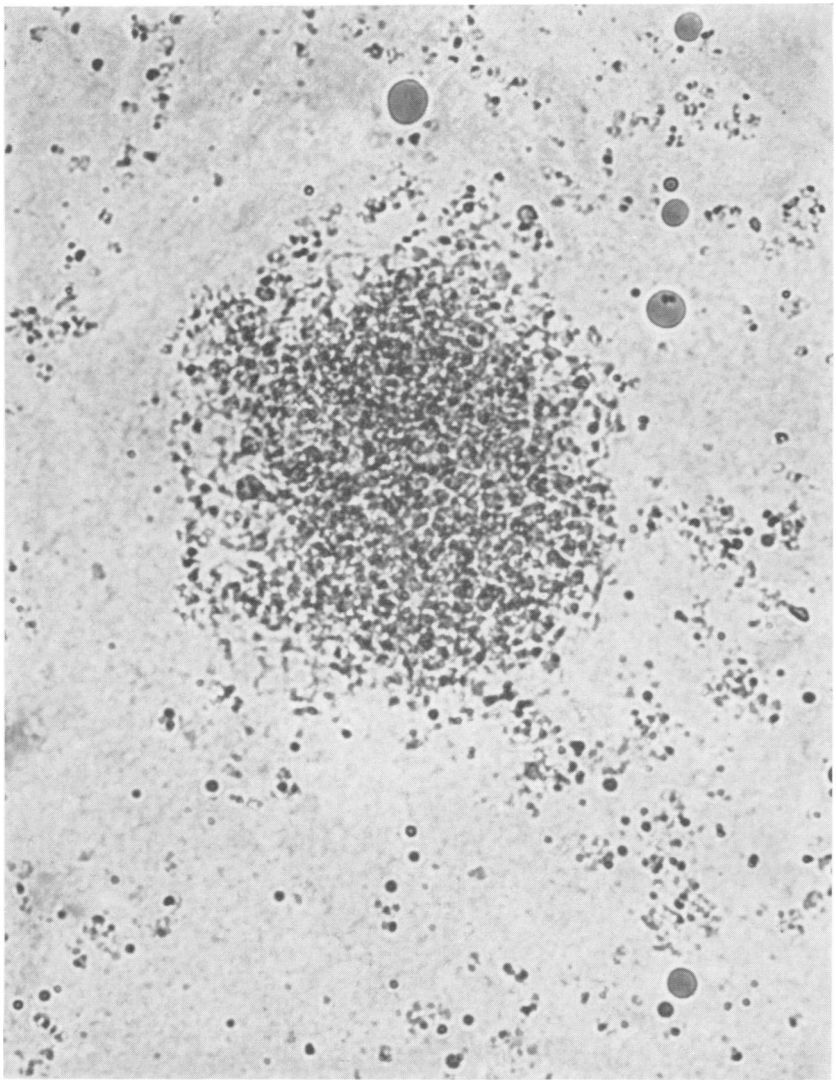

FIG. 7. "L-type" colony of an atypical bacterial form cultured from a patient with thrombophlebitis, grown on Brewer's Thioglycollate Broth with added agar (1.5%) and human plasma (12%) (Photographed at 156×).

were examined by dark-field and phase microscopy; agar cut-outs were examined by phase microscopy, Dienes' stain cover slip preparations [13] and by Gram and Giemsa stained impression smears. Specimens of clear body fluids were examined directly by dark-field and phase microscopy.

As previously reported the cultivation and identification of the various types of pleomorphic, atypical, or "L-type" growth was accomplished with considerable difficulty in cultures of 31 of 42 patients studied.[2, 6, 8] Similar forms have been recovered from an additional 19 patients studied since, making a total of 50 patients with this type of growth and coexistent thromboembolic disease. At the same time negative cultures of the blood were obtained in 41 randomized patients without thromboembolic disease who were used as controls.

Various types of morphological forms were noted in the cultures as previously

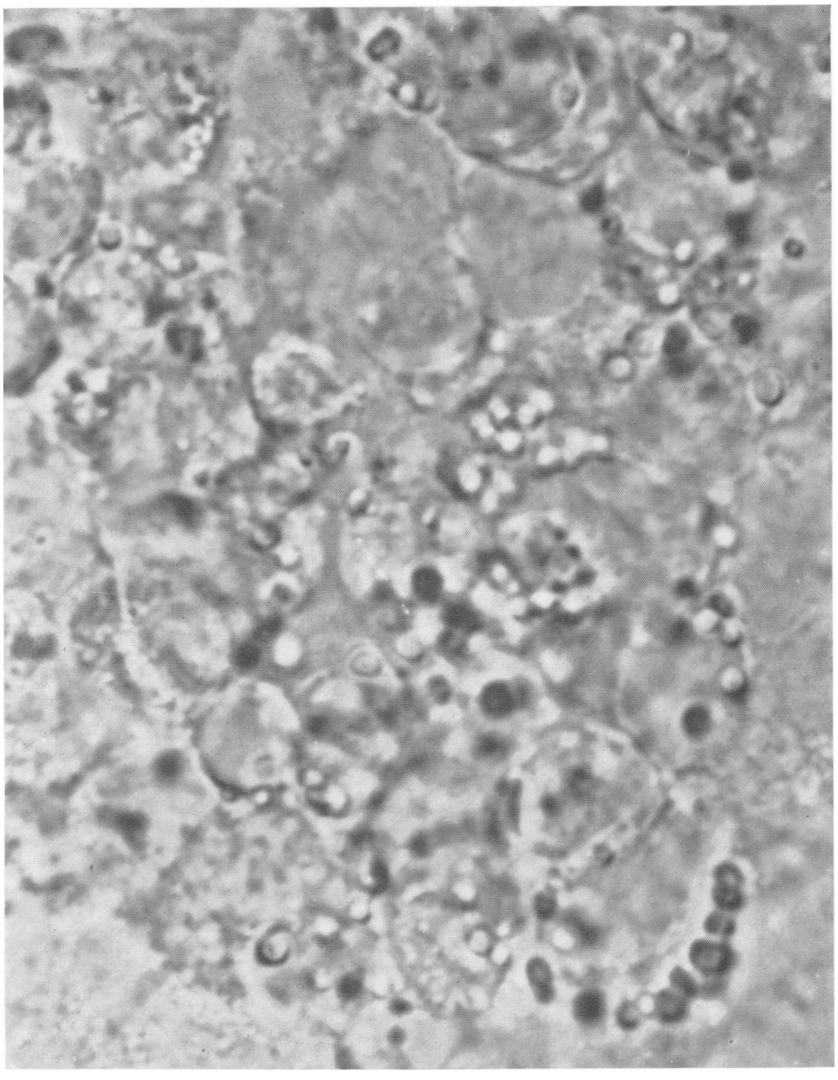

FIG. 8. A portion of colony shown in Figure 7, photographed at 1,188×. Note small budding coccoid forms, and larger "erythrocyte-like" forms.

described.[2, 6, 7, 8, 11] Forms resembling human erythrocytes with Gram-positive inclusions mimicking mitotic figures (Fig. 5) have presented an especially interesting diagnostic challenge. These may appear on direct smear as red blood cells parasitized by very small Gram-positive cocci. On two occasions these have proved to be atypical forms of a pleomorphic, Gram-negative, non-sporulating anaerobe, presumed to be *Sphaerophorus sp.* Colonies on agar plates have been of microscopic size, quite irregular and containing extremely pleomorphic forms (Figs. 6, 7, 8). The Gram-stained preparations frequently failed to demonstrate distinct morphologies, but Giemsa-stained preparations of impressive smears, and the *in situ* staining of colonies by Dienes method (Fig. 9) have proved to be much more helpful in demonstrating the small colonies which necessitate examination of agar cut-outs under magnifications of 150 to 1,200.

The occasions in which classical bacterial forms were recovered in primary culture obtained from this group of pa-

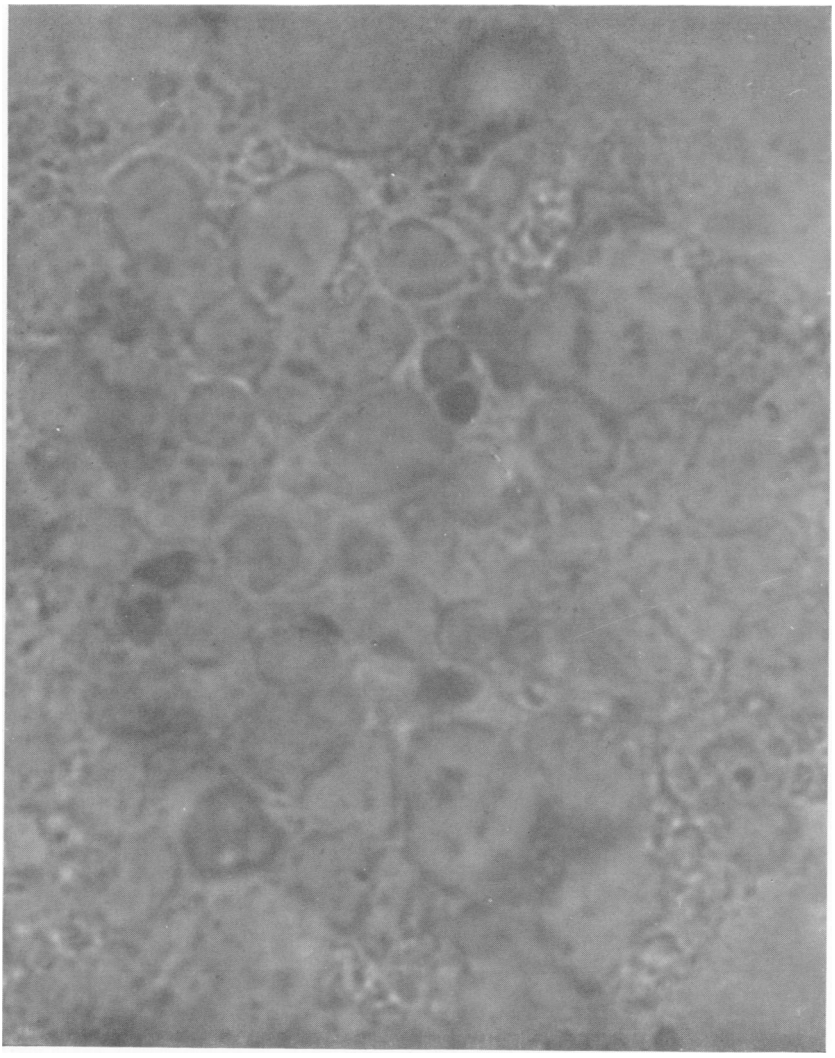

FIG. 9. Portion of an "L-type" colony of a *Fusobacterium sp.*, stained *in situ* by the Dienes method (Photographed at 1,188×).

tients have been rare. In the series reported here, only two classical bacterial forms were primarily cultured from blood and each of these was a *Sphaerophorus sp.* (Fig. 10).

Discussion

These observations on 50 patients studied during the past 6½ years have been indicative of a possible new etiologic basis of several types of acute and recurrent thromboembolic disease. At first it seemed that "L" form bacteremia was found only in patients with recurrent episodes of thromboembolic disease which often continued after vena caval ligation. Patients of this type have now been classified as Type II. While this type has been seen more commonly and has seemed to be more characteristic, two others have been noted. Type I has consisted of those in whom a single attack of thrombophlebitis and pulmonary embolism occurred, usually in the period 8 to 12 days postoperative. Type III included a smaller number of patients who had had repeated

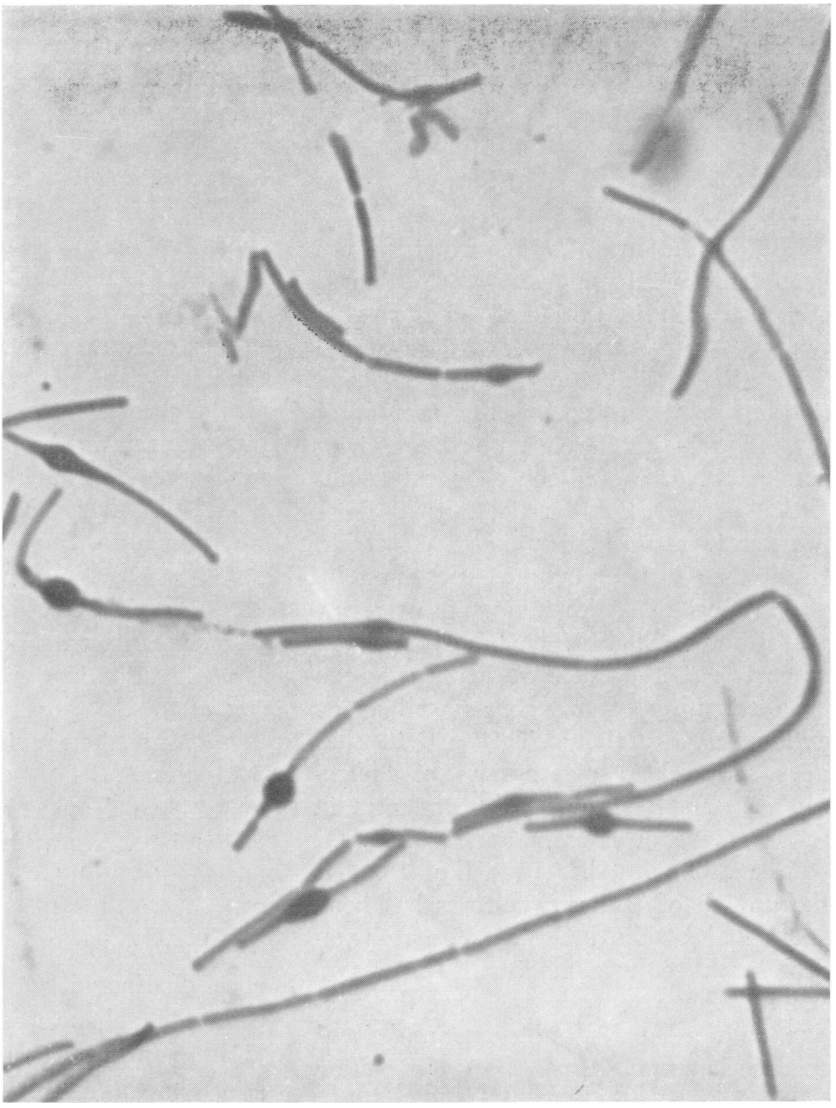

FIG. 10. Photomicrograph of filaments and funduliforms of a *Sphaerophorus sp.* recovered from a patient with thrombophlebitis (Photographed at 1,188 ×).

episodes of venous thromboembolic disease but who later developed attacks of venous thrombosis and arterial thrombosis or embolus during the same illness. The recovery and identification of "L" and other atypical bacterial forms in the blood or surgically removed thrombi of these groups of patients with thromboembolism appears to be significant.

The role of bacterial "L-forms" or atypical bacterial forms in human infections is still quite nebulous. Although there is some experimental and indirect clinical evidence that bacterial "L-forms" can be associated with disease in experimental animals and clinical patients,[10] it has not been possible to fulfill Koch's postulates with these atypical forms. Our experience in the studies reported here and our experiences in culturing "L-forms" or atypical bacterial forms from a variety of surgical infections have indicated that these were associated with various degrees of clinically manifest, distinct infections,

some of which have been fatal.[11] The report of transitional forms of *Corynbacterium acnes* in disease also indicates a distinct role of "L forms" in infections.[17]

The possible mechanism of spontaneous thrombosis induced by "L" forms has been under active investigation.

The association of "L" and atypical form bacteremia with pregnancy and Enovid administration in the female patient between 20 and 42 years of age suggested to us the possibility of a hormonal relationship which enhanced the growth of these forms, interfered with the activity of heparin, or caused the chemical degradation of heparin. Investigations of these possibilities have been pursued. Thus far, six of the "L-type" cultures recovered by us from patients with idiopathic or recurrent thrombophlebitis have demonstrated *in vitro* activity which interferes with the chemical assay of heparin as well as its anticoagulant activity. This activity has been induced in one culture of *Sphaerophorus sp.* by growing the organism in the presence of heparin and Enovid. Of additional interest has been the fact that the growth of "L-form" cultures has been stimulated by the inclusion of norethynodrel in the culture medium. Gesner and Jenkins [9] have recently reported the production of a heparinase by *Bacteroides* and this mechanism is also under investigation by us.

It would appear that we have developed now a potential model system for correlating the clinical disease of thromboembolism with *in vitro* activity of the associated organisms interfering with the blood clotting mechanism. With this tool the metabolic mechanisms involved will be studied further.

Summary

Atypical bacterial and "L" type forms have been cultured from the blood or surgically removed thrombi of 50 patients with three clinical types of thrombophlebitis or thromboembolic disease. Blood cultures in 41 control patients selected at random have been negative. Of the three clinical types described, the more common and characteristic has been the Type II in which numerous recurrent episodes of thromboembolic disease have occurred and often have continued after ligation of the vena cava.

Our data suggested that bacterial "L" type forms, particularly of anaerobic *Bacteroides,* may have played an important etiologic role in thrombophlebitis and pulmonary embolism. From the evidence accumulated thus far the sources of these bacterial forms seemed to be endogenous.

The mechanism of their action has been investigated. The association of this form of bacteremia with pregnancy and Enovid administration in many of the patients has suggested the possibility of a hormonal relationship. The evidence thus far indicates that "L" type cultures from six patients have demonstrated *in vitro* activity which interferes with the chemical assay of heparin and its anticoagulant activity. This activity has been obtained in one culture by growing the organisms in the presence of Enovid. Of further interest has been the enhancement of growth of the "L" type forms when norethylnodrel was added to the culture media.

Possibilities of making significant inroads on the prevention and treatment of thromboembolic disease have been proposed by the use of experimental models to study the mechanism of action of the "L" type forms.

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Discussion

Dr. Harold Laufman (New York): If the cell wall of bacteria is destroyed, the micro-organism will either be destroyed or a bacterial variant will be produced. These bacterial variants are of two general types. One type is described by such names as "L" forms protoplasts, spheroblasts, transitional forms or wall defective micro-organisms. There are subtle differences between each of these pleomorphs, but all represent wall defective variants which will revert to the parent bacterium either *in vitro* or *in vivo*. Some are gram-positive; others gram-negative, and still others gram-variable.

The second type, the microplasma species also lack a rigid wall, and morphologically resemble "L" forms, but are immunologically distinct, and although they may also once have derived from bacteria, they have never been shown to have a proved relationship with any other bacterial organism.

My interest in "L" forms began when we were doing some investigative work in the field of pulmonary embolism, a number of years ago at Northwestern University Medical School in Chicago. We found, as did others, that microplasma and "L" forms were responsible for atypical pneumonia in some postoperative patients. In those days we failed to distinguish between the types of variants, and we used the old terminology of PPLO or pleuro-pneumonia-like organisms to describe all of them.

Recently, my interest was revived when our microbiologist at Montefiore Hospital, New York—Dr. Jacques Singer—isolated an "L" form from the blood of a patient who had recurrent fever after being treated with penicillin for vegetative valvular disease.

Wall-deficient bacterial forms may be induced by antibiotics with an action directed against cell-wall formation. Penicillin and methycillin are such antibiotics. Cell-deficient variants may also be caused *in vivo* by enzymes, by nutrients deficient