PROTOTHECOSIS, AN ALGAL INFECTION: REPORT OF A CASE IN MAN

G. K. KLINTWORTH, B. F. FETTER AND H. S. NIELSEN, JR

*Departments of Pathology and Microbiology, Duke University Medical Center,
Durham, North Carolina, USA*

PLATES XVII-XXI

SINCE the establishment of the causal relation between micro-organisms and infectious diseases, a wide variety of agents have been found to be pathogenic. However, algae have seldom been associated with disease, and rarely incriminated as the causal agent of lesions. It was not until 1964 that the first proven case of human infection by an alga was documented. Davies, Spencer and Wakelin (1964) not only isolated the achloric alga *Prototheca segbwema* from a cutaneous lesion of a rice farmer in Sierra Leone, but clearly demonstrated the organisms within the tissue. In their patient, the alga subsequently spread to the regional lymph-nodes (Davies and Wilkinson, 1967). The present paper reports the second instance of human infection by *Prototheca* in which the organism was cultured from and demonstrated in the lesions. These were ulcerating papules of the skin of the lower leg.

CLINICAL FINDINGS

A 45-yr-old Caucasian woman from Salisbury, North Carolina, developed tender cutaneous lesions of a papulopustular variety on the distal portion of the leg about December 1966. In April 1959 the patient had undergone a radical mastectomy for carcinoma of the breast. This was followed by a period of good health until July 1963, at which time sternal pain developed. In December 1963 metastatic adenocarcinoma was detected in the lower end of the manubrium sterni and osteolytic lesions were noted in the cervical spine. She was admitted to hospital many times for symptoms due to metastatic carcinoma, particularly in bones.

Her illness was complicated by attacks of herpes zoster of the right arm, shoulder and upper back in August 1964 and April 1966, by oral candidosis in April 1967, and by urinary tract infections with *Escherichia coli* and *Aerobacter* at various times in the period 1964-67. Diabetes mellitus, hirsutism and a Cushingoid appearance also developed. Therapy included bilateral oophorectomy and administration of androgens, corticosteroids, and cytotoxic and other drugs (fig. 1). Localised radiotherapy was administered to the sternum, lower ribs, cervical spine, lower lumbar spine, right hip, right femur and pelvis.

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The haematocrit, haemoglobin and leucocyte count were followed regularly from 1964 and were usually within normal limits. In November 1966 the leucocyte count, haematocrit value and haemoglobin value dropped temporarily to 2000 per mm³, 36 per cent. and 11.3 g per 100 ml, respectively.

FIG. 1.—Diagram summarising the prominent features of the patient's therapy and the course of her illness after the detection of carcinoma of the breast. (1) Radical mastectomy. (2) Metastatic carcinoma in bone. (3) Bilateral oophorectomy. (4) Onset of diabetes mellitus. (5) Lesions appeared on legs.

Examination of the patient revealed a cutaneous eruption consisting of about a dozen crusted papular lesions over the anterior, lateral and medial aspects of the left lower leg. Most individual lesions measured 2–5 mm in diameter, but a few were as small as 1–2 mm. The lesions were characterised at different stages of their development by ulceration, a small amount of purulent discharge, umbilication and crusting. Removal of the crusts revealed tiny depressed ulcers with slightly inflamed bases. The superficial regional lymph-nodes were not enlarged.

HISTOLOGICAL METHODS

Punch biopsies were performed on three separate occasions on three different lesions on the leg. Part of the tissue was fixed in Zenker's fluid, dehydrated, and embedded in paraffin for light microscopy. Histological sections were stained with haematoxylin and eosin, Grocott's methenamine silver (Grocott, 1955), Mowry's modification of Hale's colloidal iron technique (Mowry, 1958), modified periodic acid-Schiff stain (Kligman, Mescon and DeLamater, 1951), bacterial stains (MacCallum, 1919; Brown and Brenn, 1931) and acridine orange (Pickett *et al.*, 1960).

Parts of the lesions were sectioned into small cubes measuring less than 2 mm. These were immediately fixed either in cold veronal-buffered 2 per cent. osmium tetroxide (pH 7.4) for 1 hr, or in cold sodium cacodylate-buffered glutaraldehyde (pH 7.4) for ½–2 hr, and then post-fixed in 2 per cent. osmic acid for 1 hr. All were dehydrated in graded alcohols,

infiltrated with styrene and embedded in Epon. Thin sections were cut with a Serval Porter-Blum microtome, mounted on 300-mesh copper grids and stained for 10 min. in lead citrate at a high pH at room temperature (Reynolds, 1963). Electron-microscope examinations were made with an RCA EMU-3G microscope at 50 kV. Companion sections cut at 2 μ m were stained with thionine-azure blue for purposes of orientation (Tzitsikas, Rdzok and Vatter, 1961).

Portions of biopsied material were inoculated on to blood agar and brain heart infusion cystine agar, and incubated aerobically and anaerobically at room temperature. Similar specimens were macerated in a tissue homogeniser and saline suspensions were inoculated intraperitoneally into guinea-pigs and mice.

MICROBIOLOGICAL FINDINGS

An organism identified as *Prototheca wickerhamii* was cultured from two different lesions on separate occasions. It grew readily on blood agar, Sabouraud's glucose peptone agar and brain heart infusion cystine agar, and produced a smooth moist cream-coloured colony similar to that of a yeast. No other organism was cultured.

The isolate fermented glucose with the production of acid only, but did not ferment other common laboratory sugars. Galactose, glucose and mannose, as well as ethanol and glycerol, were readily assimilated. Sparse growth was observed on media containing methanol, propionic acid or sodium acetate, but a number of other carbon-containing compounds were not utilised.

The alga grew poorly below 20°C and was completely inhibited at a temperature of 38°C or above. The optimum temperature for growth at pH 7.0 appeared to lie between 30° and 32°C. Essentially no growth occurred at pH levels above 7.0. Tests on a medium containing vitamin-free casamino acids, basal salts and 1 per cent. glucose showed that the isolate had a requirement for thiamine, a property characteristic of all species of *Prototheca*.

Light-microscope findings

The lesions consist predominantly of abundant, closely packed round or ovoid organisms, which extend to a depth of up to 5 mm below the cutaneous surface. Intermingled with the algae are small foci of necrotic debris (fig. 2). The overlying epidermis shows hyperkeratosis, slight parakeratosis and, in some areas, focal ulceration. The cellular reaction to the organisms is minimal and consists chiefly of a few neutrophils, lymphocytes and macrophages. A small amount of fibrosis is present at the periphery of some lesions. The algal cells vary markedly in size, from 2 to 11 μ m in diameter, and the larger organisms have thick walls and characteristic internal septations (fig. 3). Many, particularly those lying within macrophages, are in different stages of degeneration. The algae are basophilic and Gram-positive, and stain well with Grocott's methenamine silver, Mowry's modification of Hale's colloidal iron method, and the modified periodic acid-Schiff technique. The organisms show secondary fluorescence when stained with acridine orange. The morphological attributes of the isolate are essentially identical with those of the alga in the tissues (fig. 4). Neither neoplastic cells nor other organisms are identified in the lesions.

Electron-microscope findings

Cells of the alga cultured on Sabouraud's medium regularly contain nuclei, coarsely granular material, vesicles of different size and constituents, and various cytoplasmic organelles. Plastids and lamellar photosynthetic structures comparable to those of autotrophs are not present (fig. 6). The cell wall varies with the size of the alga, being 32 nm thick in the smallest and 163 nm thick in the largest organisms. The larger organisms tend to be more electron dense than the smaller ones and contain an inconstant number of sporangiospores, which are variable and often irregular in shape (fig. 7). Occasionally, as many as twelve daughter cells are present in a single plane of section. Calculations based on the dimensions of the sporangiospores and the parent cells indicate that the latter each contain approximately 50 sporangiospores. Multinucleated daughter cells are not observed, strongly suggesting that cytoplasmic cleavage follows soon after nuclear division. In this regard *Prototheca* differs from the ascomycetes, in which no cytoplasmic cleavage occurs until the succession of nuclear divisions is complete.

Ultrastructural studies of the lesions reveal numerous intracellular and extracellular organisms, with fine structural attributes similar to those of the isolate. In tissue, however, the organisms are more commonly present in various stages of degeneration (figs. 8 and 9). No other organisms are observed in the lesions by electron microscopy.

Pathogenicity

Subcutaneous and intraperitoneal inoculations of saline suspensions of organisms failed to produce disease in guinea-pigs, mice, rats and rabbits. The organism also was not pathogenic when instilled into the anterior chamber of rabbits' eyes.

DISCUSSION

The genus *Prototheca* was introduced in 1894 by Krüger to designate a group of unicellular organisms isolated from the mucous flux of trees. This alga resembles achloric mutants of *Chlorella* in its morphology and mode of reproduction; it differs, however, from these mutants in requiring thiamine for growth. The organism begins cell division before maturation, and division continues until numerous daughter cells are present. The latter are liberated after rupture of the parent structure and increase in size before passing again through the life cycle (fig. 10). Seven species of *Prototheca* are currently recognised: *P. ciferrii*, *P. moriformis*, *P. portoricensis*, *P. segbwema* (fig. 5), *P. trispora*, *P. wickerhamii* and *P. zopfii*. No fundamental physiological differences have been observed among the accepted species, which are presently distinguished by their sizes (Tubaki and Soneda, 1959).

Despite the fact that *Prototheca* has been isolated from lesions of only one patient in whom it was demonstrated (Davies *et al.*, 1964; Davies and Wilkinson, 1967), it has been cultured from fingernails, faeces and sputum (Ashford, Ciferri

and Dalmau, 1930, and unpublished observations communicated to us by Drs D. L. Adler and D. G. Ahearn), a cutaneous lesion of the thumb (Gordon, 1966) and milk obtained from a cow with mastitis (Ainsworth and Auswick, 1955). In addition, organisms believed to be *Prototheca* have been observed in, but not cultured from, superficial cutaneous and bursal lesions in a man (unpublished observations of Drs D. G. Ahearn, C. W. Emmons and M. Millard), ocular and widely disseminated lesions in a dog (unpublished observations of Drs C. W. Emmons and F. M. Garner), and gastro-intestinal lesions in a cow (unpublished observations of Dr C. H. Binford). In most instances, the alga has been regarded as either a saprophyte or a secondary invader.

In view of the close morphological similarity, there can be little doubt that the organisms cultured from our patient were identical with those seen in her lesions. The relation between the alga and our patient is thought to be neither a symbiotic nor a commensal one. *Prototheca* was most certainly pathogenic in the present case—as it was in the case described by Davies *et al.* and Davies and Wilkinson—since (1) the lesions containing the alga involved previously normal tissues, (2) the organisms were present to a depth of up to 5 mm into the tissues, and (3) the alga was the only organism found in the lesions.

At present one can only speculate about the conditions that are conducive to infection with *Prototheca*. The development of oral candidosis and two episodes of herpes zoster during the course of therapy suggest that the patient's general resistance to infection was low. Diabetes mellitus predisposes to infection and may have been a contributing factor. There is ample evidence from both experimental and clinical studies to support the view that therapeutic regimens, which include cytotoxic drugs, antibiotics and steroids, predispose to infection by a diversity of micro-organisms. Some drugs appear, either alone, or in combination with an associated debilitating disease, to alter natural defences. Particularly in recent years, it has become apparent that a wide variety of microbes, heretofore considered non-pathogenic, produce spontaneous disease in man under particular circumstances. This phenomenon of microbial opportunism has been observed in infections by bacteria, viruses, fungi and protozoa. The failure, thus far, to reproduce the disease in animals by inoculation of the algal isolate argues in favour of the hypothesis that *Prototheca* ordinarily requires a distinct, but as yet undefined, alteration in the host resistance before it can act as a pathogen.

SUMMARY

A case of cutaneous human infection with a species of the achloric algal genus *Prototheca* is described. The infection occurred in a woman who had diabetes mellitus and widespread metastatic carcinoma from the breast, and whose therapy included corticosteroids, cytotoxic drugs, antibacterial agents and antibiotics. The organism was cultured from the lesions and identified in them with the light and electron microscopes on two separate occasions. It was non-pathogenic on inoculation in guinea-pigs, mice, rats and rabbits. The case is viewed in the light of the concept of microbial opportunism, and it is

postulated that the alga requires a distinct, but as yet undefined, alteration in the host resistance before it is able to act as a pathogen.

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FIG. 2.—The algae (black dots) in this cutaneous ulcer extend to a depth of 5 mm. Grocott's methenamine silver (MS). $\times 75$.

FIG. 3.—Prototheca cells, including a mature sporangium (arrows), are shown in the lesion at high magnification. Modified periodic acid-Schiff (PAS). $\times 1000$.

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FIG. 4.—Photomicrograph of the cultured prototheca after it had been embedded in paraffin and sectioned. Note the similarity of the isolated organisms to those in tissue (fig. 3). MS. $\times 1000$.

FIG. 5.—The species of *Prototheca* isolated by Davies, Spencer and Wakelin (*P. segbwema*) is larger than that from the present case. (Photograph of a histological slide supplied by Dr R. R. Davies.) PAS. $\times 1000$.

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FIG. 6.—Electron micrograph (EM) showing an immature form from the culture of *Prototheca*. Nucleus (N), mitochondrion (M), intracytoplasmic vesicle (V), granular material (G). Lead citrate (LC). $\times 33,600$.

FIG. 7.—Mature sporangium containing several sporangiospores in a single plane of section. EM, LC. $\times 9270$.

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FIG. 8.—Protothecal cell with sporangiospores in the cytoplasm of a macrophage. EM, LC. $\times 11,400$.

FIG. 9.—Portions of the thick cell wall of a mature sporangium in the cytoplasm of a macrophage. EM, LC. $\times 10,900$.

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FIG. 10.—Schematic reconstruction of the inferred life cycle of the alga isolated from the patient.