with filter). At intervals the suspension was plated out and incubated. Colonies were subcultured into broth and checked for presence of chains.

Irradiation for 15 min. reduced the number of colonies substantially; 60 min. failed to sterilise the suspension completely and one or two colonies invariably survived. Colonies from the irradiated suspensions initially differed in no way from controls, and fluid cultures obtained from them consisted of single cocci or pairs of cocci. However, when the plates, after initial incubation at 37°C, were kept at room temperature for 3 wk, the colonies from irradiated suspensions became strikingly different from the colonies on the control plates. They grew in size up to 12 mm, and a proportion of them developed daughter colonies. Some developed "outbursting wedges" reminiscent of those described by Shinn (1939). Some of the wedges, growing out several millimetres from the periphery of the colony, showed brush-like protrusions. When subculture was made into broth from the distal ends of the wedges, long-chaining cultures were obtained. Filtrates of short-chain cultures broke down the chains of the mutants, but filtrates of the long-chain cultures were devoid of unchaining ability.

Several long-chain mutants were obtained from each strain in every experiment. All came from suspensions irradiated between 20 and 40 min. in which the percentage kill was well over 90 per cent. Out of several hundred control plates representing well over 50,000 colonies, once only was a colony with a "wedge" observed; subculture from it gave rise to long chains in broth.

One long-chain mutant reverted after 2 mth to the short-chain form, but the remaining mutants have so far proved stable. It is at present impossible to decide whether irradiation selected the long-chain mutants or led to their production, but we believe the latter to be the case. The results we have obtained are in keeping with the hypothesis that the disaggregation of chains of streptococci is due to a specific enzyme activity that may be lost by mutation.

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DISSEMINATED CANDIDOSIS FOLLOWING AORTIC VALVE HOMOGRAFT REPLACEMENT AND TRACHEOSTOMY

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PLATE XXII

THE incidence of disseminated candidosis in reported cases is limited almost entirely to the period following the introduction of antibiotics, chemotherapy and adrenal hormones (Zimmerman, 1955; Keye and Magee, 1956; Louria, Stiff and Bennett, 1962; Symmers, 1964). The diagnosis is often difficult since the significance of a finding of candida in blood,

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DISSEMINATED CANDIDOSIS

FIG. 1.—Lesion in lung showing prolific growth of fungus in a small vessel. Methenamine silver. $\times 800$.

FIG. 2.—Cerebral cortex showing mycelium in necrotic tissue. MS. $\times 800$.

FIG. 3.—Renal tubules showing Y- and M-forms of candida. MS. $\times 800$.

sputum and urine is not always clear (Kozinn and Taschdjian, 1966) and the clinical signs and symptoms are non-specific (Louria *et al.*). Furthermore, the opportunities for culturing the organism at necropsy are often missed and the fungus does not show up well in sections stained with haematoxylin and eosin.

The present case is unusual for two reasons: (1) the infection was studied in its early stages, when several observations relevant to its pathogenesis were made; (2) the infection was not suspected clinically or at necropsy.

CASE REPORT

F.G., a male nurse, was first diagnosed as having calcific aortic stenosis with incompetence at the age of 54. Three years later, on 27 Aug. 1966, aortic valve replacement was carried out under hypothermia. The myocardium was perfused via cannulated coronary arteries for 180 min. After the operation the patient was hypotensive and oliguric, and required mechanical ventilation through a tracheostomy. He developed atrial fibrillation and became jaundiced. He received numerous antimicrobials: before his operation, tetracycline and neomycin, and after it, cloxacillin and ampicillin for 9 days, then cloxacillin and BRL 2064 for the 5 days preceding death. *Candida* organisms were grown from blood taken on two occasions on the 8th day. From the sputum, *Escherichia coli*, another coliform bacillus and a *Candida* organism were grown on the 10th day, and *E. coli* and a *Candida* organism were grown on the 11th and 13th days. The results of the blood cultures yielding *Candida* were not known until after death.

Necropsy and histological findings

The immediate cause of death was a haemorrhage into the outer media of the descending branch of the left coronary artery, i.e., it was a localised dissecting aneurysm probably caused by trauma. The left ventricle showed a large infarct in its anterior wall with surrounding partial infarction. This lesion corresponded in age with the survival time of 13 days. The myocardium elsewhere showed the irregular necrosis of muscle fibres described by Morales, Fine and Taber (1967) in the "low output syndrome following cardiac surgery", but calcific emboli were absent. The homograft valve was clear of vegetations, though microscopically it was covered by a thin deposit of fibrin and was being endothelialised. The mitral valve showed the changes of chronic rheumatic valvular disease. Around the tracheostomy, three cartilages were ulcerated with fibrinous lesions. The lungs contained several firm lesions. The brain showed many cortical softenings, largely compound granular corpuscles. The liver contained scattered micro-abscesses and there were acute inflammatory foci in renal glomeruli and tubules.

Identification of fungus

The fungus was grown on blood agar and Sabouraud's medium. It was identified only as a species of *Candida*. Histologically, no fungi are seen in the initial sections, which were stained with haematoxylin and eosin, and only become obvious with special stains. The periodic acid-Schiff method shows them well, but methenamine silver is even more helpful. Understaining with PAS and overstaining with silver was avoided. Round and oval blastospores (Y forms) and mycelium (M forms) were identified. According to Mackenzie (1966) *Candida albicans* is considered to be the only species to form abundant mycelium *in vivo*. Two main criteria are commonly used in assessing the pathological significance of fungi seen histologically: (1) the presence of the fungus in sites where it is normally absent, e.g., blood, cerebrospinal fluid, skin and centres of inflammation, is taken as indicating that it has a pathogenic role; (2) the presence of mycelium is similarly taken to indicate pathogenic activity since most authors consider the filamentous forms to be the main agents of tissue invasion (Kozinn and Taschdjian; Mackenzie).

Distribution of fungus

The density and morphology of the fungus infection vary at different sites. Thus, filamentous forms are scanty in some of the candida lesions around the tracheostomy site, but form the bulk of the massive growth in the ulcerated cartilages. A thrombosed vein nearby contains a single mycelial form. The lone infected pulmonary lesion shows a massive growth of both yeast and filamentous forms extending from arterioles to venules and from septal capillaries into alveolar exudate (fig. 1).

The homograft valve shows no signs of invasion by fungus, though scanty Y and M forms are seen in the surface of a fibrinous deposit. Only one of the cerebral infarcts contains fungus; this is in the filamentous form (fig. 2). Fungi are frequently seen in capillaries in kidney and liver, in the absence of any inflammatory reaction. The renal glomeruli contain Y forms, whereas free fungi of both yeast and filamentous types are present, apparently as a result of capillary rupture, in intact renal tubules (fig. 3).

DISCUSSION

In explaining the pathogenesis of this infection the following observations must be taken into account: (1) the extensive growth of candida around the tracheostomy site and in one of the pulmonary lesions, (2) the minimal valvulitis, and (3) the scattered infected lesions in brain, kidney and liver. It is likely that the initial spread was thrombo-embolic from the candida lesion around the tracheostomy and that this spread produced multiple lesions in the lungs, only one of which was infected with *Candida albicans*.

The fungaemia may have arisen from three sources: (1) direct from the tracheostomy site, (2) from the infected pulmonary lesion, and (3) from the homograft valve. A single fungal filament was demonstrated in the thrombosed veins draining the tracheostomy region. However, pulmonary capillaries are relatively large and may allow individual fungal elements to pass without causing lesions (Louria *et al.*, 1962), and, in this case, the capillaries were passively distended, so that the possibility of direct spread from the tracheostomy site into the systemic vascular system cannot be entirely excluded. Infection of the homograft valve was slight and recent, indicating that it was probably a complication rather than a cause of the fungaemia. Taking all factors into account it seems most likely that the infected pulmonary focus gave rise to the blood-borne fungus. Growth of candida in the lesion was prolific and extended from the embolised small arteries into the venules, so that the further spread into the circulating blood could easily have taken place. The brain lesions may have been produced by more than one mechanism: either by embolism with uninfected clot from the fibrillating left auricle or by infected thrombo-embolus from the lung.

The form taken by the fungus may reflect local conditions rather than its virulence. Round forms are usually found in the circulating blood, possibly because these forms are more easily disseminated and are favoured by a high oxygen tension. Thus, free round forms were plentiful in the renal capillaries, but mycelial forms were more prominent in the necrotic tissue in brain and in the renal tubules.

SUMMARY

A tracheostomy following aortic valve homograft replacement was infected with organisms of a *Candida* species. The infection spread to one of many thrombo-embolic lesions in the lung and became fungaemic with production of widespread microscopic foci. Infection of the homograft valve was minimal and secondary to the fungaemia.

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