test[3], and Saccharomyces cerevisiae Hansen for the paper disk method[3]. The level of antifungal activity was determined by preparing a 2fold dilution series of each methanol extract and assaying them for their inhibitory effect on conidial germination of the fungi and the growth of the yeast on paper disks.

Leaves of those species which contained antifungal activity in methanol extracts were then extracted sequentially with n-hexane, benzene, ethyl acetate and butanol. Extracts of each solvent were concentrated in a vacuum evaporator, suspended in deionized water and assayed for antifungal activity using the C. miyabeanus conidial germination test method.

Extracts of the above which showed antifungal activity were examined further by thin layer chromatography. R_f-values of the various components were measured and their antifungal activities were determined by testing methanol extracts of each component against C. miyabeanus.

Results and discussion. Antifungal activities detected in methanol extracts of the various species of Eucalyptus are shown in table 1. 2 species contained antifungal substances to A. solani, 10 species to C. miyabeanus, and 2 species to S. cerevisiae. 8 species contained antifungal substances which could be extracted by various solvents, indicating that there was probably more than 1 kind of component involved. These extracts (except those from the butanol and residual layers) were further separated by thin layer chromatography and found to consist of from 1 to 4 different components (table 2). At present, we do not know if these components are the same as or similar to those isolated from E. gunnii.

Myofibroblasts in hepatic schistosomal fibrosis[1]

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Summary. Myofibroblasts were identified in liver portal spaces of patients with Symmers' fibrosis following infection by Schistosoma mansoni.

In chronic human schistosomiasis the liver is always involved by intense fibrosis of multiple aspects. In severe and long-standing infections, classical Symmers' fibrosis develops. The principal characteristic of this lesion is scar-like fibrotic thickening of main portal spaces, some of which are joined with the Glisson's capsule forming deep furrows of sclerotic tissue at the surface of the liver[2]. Although this elementary lesion is one of the most frequent in tropical pathology, the cells implicated in its histogenesis are still little known.

Materials and methods. 32 surgical or percutaneous needle biopsies were taken from livers of patients chronically infected by Schistosoma mansoni. Patients were described in earlier publications[3,4]. Large portal spaces were identified macroscopically and fixed separately for this study. Small fragments of fresh tissue were fixed and treated by standard histological methods for optical microscopy. For electron microscopy, double glutaraldehyde/osmic fixation of simple osmic fixation were used, followed by dehydration in ethanol and propylene-oxyde and embedding in Epoxy resin. Semi-thin sections were treated by Richardson's technique, and examined for each fragment studied in electron microscopy.

Results. At a histological level, the main portal spaces were characterized by the following pathological modifications: granulomatous inflammatory reaction to schistosomal eggs, chronic endophlebitis and periphlebitis, progressive obliteration of portal vein with secondary angiomatous proliferation and progressive arterialization of portal liver circulation, moderate ductular proliferation, diffuse mononuclear and lymphoid infiltration and intense proliferation of connective tissue.

In the connective tissue, our attention was attracted to 3 morphologically similar cells. Classical fibroblasts were very frequent, embedded in dense collagen deposits, where they formed a loose and regular network. They were characterized by stellate form and elongated nuclei. In places where obliterated portal vein was located, large parallel bundles of cells were observed. They retained characteristics of smooth muscle cells, and they often had a circular distribution corresponding to the obliterated blood vessel. In certain regions of portal spaces, groups of elongated fusiform fibroblast-like cells were observed. They had morphological characteristics of classical fibroblasts but they could be distinguished by their regular fusiform shape and by their close cell-to-cell contacts, as they were tightly packed and arranged in long strands parallel to the main portal axis.

At the ultrastructural level, this last type of cells was identified as typical myofibroblasts[5] (figure 1). This identification was based on the following morphological characteristics, observed in portal spaces of all biopsies of schistosome-infected livers. Myofibroblasts were spindle-shaped with central elongated nucleus. The nuclei often showed indentations or folds, although this feature was less frequent than is classically described in scar tissues. Chromatin distribution in myofibroblasts in homogenous and dense chromatin patches was limited to a narrow zone underlying the nuclear membrane. Small nucleoli were often present. In the cytoplasm, rough endoplasmic reticulum and mitochondria were less developed than in normal fibroblasts. Golgi was always present and well developed (figure 1). A fibrillar system was always present in the cytoplasm, particularly in the regions subjacent to the cellular membrane (figure 2). The fibrils were arranged in groups, parallel to the elongated cell axis. Electron dense areas were scattered in the cytoplasm, most frequently at the periphery of the cell, immediately beneath the plasmalemma. At the same position, extremely numerous pinocytic vacuoles were observed, remarkably similar in size in all myofibroblasts, their diameter measuring 0.01–0.02 μm.

One of the most characteristic features of myofibroblasts was the presence of a well defined layer of material ressembling a basal membrane (figures 1 and 2). Often, this layer appeared as large tufts of fibrillar structures and

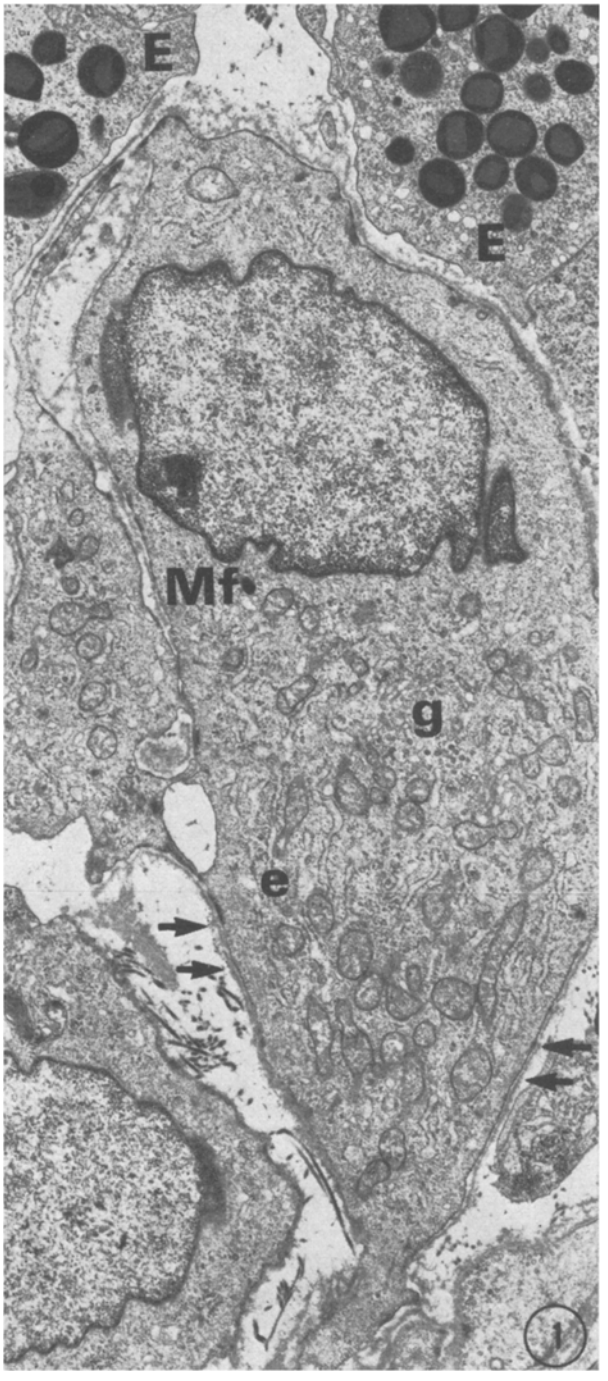

Fig. 1. Myofibroblast in portal space (human schistosomal fibrosis). Mf, myofibroblast; E, eosinophil granulocyte; G, Golgi; e, ergastoplasm; ⇉, basal membrane. ×7700.

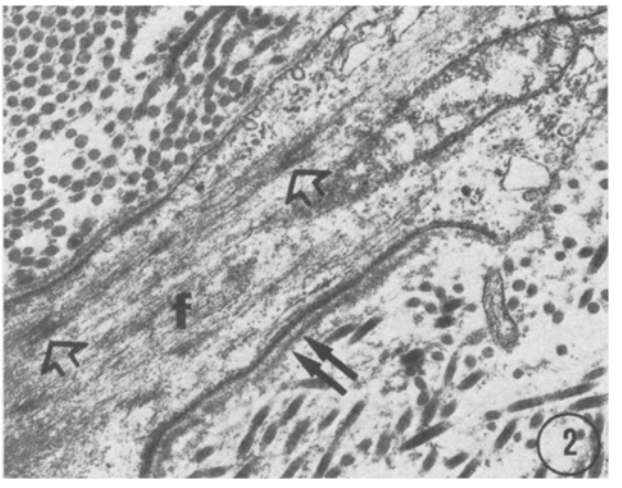

Fig. 2. Detail of myofibroblast cytoplasm. f, intracytoplasmic fibrils; ⇒, electron dense areas; ⇉, basal membrane. ×22,000.

Characterization of cell types in fibrotic portal tissue in Human schistosomiasis

	Fibroblasts	Myofibroblasts	Smooth muscle cells
Indentations and folds in nuclei	–	*	*
Nucleoli	*	*	–
Ergastoplasm	***	**	*
Mitochondria	**	**	*
Golgi	**	*	–
Myofibrils	–	*	***
Cytoplasmic densifications	–	*	**
Basement membrane	–	*	*
Intercellular contact differenciation	–	*	**

*** Very prominent. ** Prominent. * Present. – Rare or absent.

became intermingled with neighbouring collagen fibres. Intercellular connections between myofibroblasts were characterized by bilateral membranar densifications (figure 3). The morphological characteristics of fibroblasts, myofibroblasts and smooth muscle cells, observed in portal spaces of schistosomal livers, are summarized on the table.

Discussion. The myofibroblasts observed were well characterized and identical to those seen in hypertrophic scars and other granulation tissues by other authors[5–10] and to those observed in the connective tissue of Dupuy-

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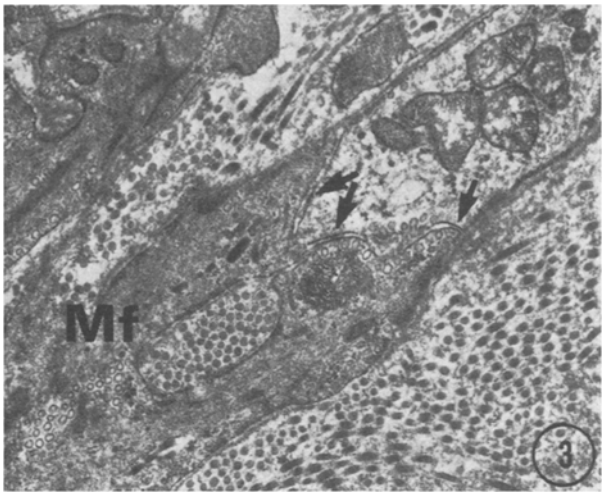

Fig. 3. Intercellular contacts (→) between 2 myofibroblasts (Mf). ×15,500.

tren's contracture[12]. Their surprising position in liver portal spaces of patients infected by Schistosoma mansoni may be related to 2 different mechanisms.

Portal spaces are involved in chronic human schistosomiasis by long standing and intense inflammatory reaction corresponding to chronic irritation caused by metabolic products and eggs eliminated by worms into the portal circulation. This inflammation may stimulate an intense proliferation of fibroblasts, with subsequent modification of their differentiation into contractile cells[9, 11], as was proposed by Gabbiani et al. in cases of hypertrophic scars[5, 10].

At the same time, vascular walls are submitted in schistosomiasis to chronic injury, resulting in inflammation and in their progressive degeneration. This lesion is a constant characteristic of chronic human schistosomiasis and precedes the development of Symmers' fibrosis. The degenerated smooth muscles of vascular walls may thus be the origin of myofibroblasts. This latter hypothesis appears to us as more plausible, but further investigations are necessary to elucidate the origin of myofibroblasts in this model.

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Piroplasmal sporozoites in the Argasid Ornithodoros erraticus (Lucas)

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Summary. The infective forms of Nuttallia meri, a small-mammal piroplasm, first appear as spindle-shaped sporozoites in and around the salivary glands of Ornithodoros erraticus (small race) 30 days after the ticks had fed on infected blood. O. erraticus is the only soft tick so far proved to transmit a piroplasm.

Nuttallia meri Gunders, 1971, was isolated and described from the fat sand rat Psammomys obesus Cretzchmar, 1828, from the lower Jordan Valley[2] and is considered a potential model for the study of piroplasmosis of economic importance. The search for possible tick vectors in nature led to the discovery, within Psammomys burrows, of only the 'soft' tick Ornithodoros erraticus (Lucas, 1849, small race).

We report here on the finding of the infective form of N. meri in the salivary glands of its natural vector.

Material and methods. Clean laboratory raised Ornithodoros erraticus (Lucas, small race), were fed on Psammomys obesus Cretzchmar 1828, or hamsters infected with N. meri. The gorged ticks were kept in a darkened incubator at 28 °C and about 80% RH (relative humidity). Ticks were dissected under a binocular stereomicroscope at different times after repletion, when salivary glands and stomachs were removed on to clean microscope slides lightly rubbed with a silicone cream, and covered. A mushroom-like weight was used to flatten the preparation which was then fixed in Bouin and stained with Giemsa stain (1:15), dehydrated and mounted.

Results and discussion. Spindle-shaped slender sporozoites were first seen in glands removed 30 days after the infective feed, and up to 85 days later (no dissections were carried out after this). The sporozoite cytoplasm stained a faint blue, and the usually central nucleus a dark red; they measured 3–4 μm (median 3.4) in length × 1 μm. A small peri-nuclear vacuole was seen in most. Immature,

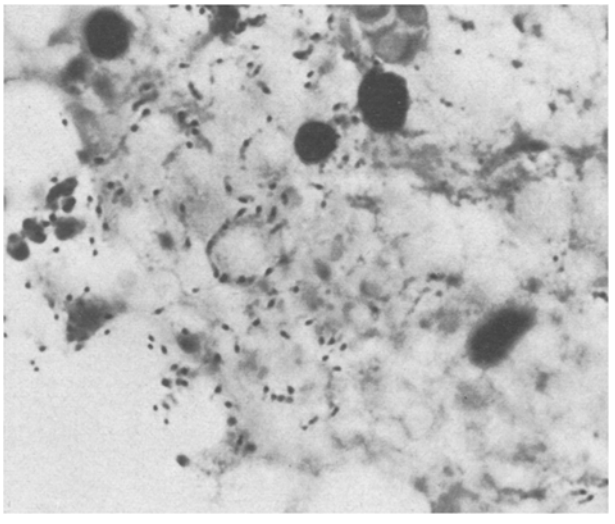

Fig. 1. Periphery of flattened salivary gland of Ornithodoros erraticus (small race) with discrete sporozoites of Nuttallia meri. Giemsa-stained, ×1200.