sections and counted for radioactivity. The mobilities of the didymis were radioiodinated, extracted with Triton X-100 esis in a mannoside. The mobilities of the H- and L-chains of y-acrylamide-gel. The electropherograms were cut into 1 mm (.---a), 442 and fractionated by electrophoresis on an 11% SDS/polyacrylamide-gel of that fraction of a (A-A), (A--A), corpus (---) and cauda ( - - ) epidermydis were radioiodinated, extracted with Triton X-100 and fractionated by electrophoresis on an 11% SDS/polyacrylamide-gel. The electropherograms were cut into 1 mm sections and counted for radioactivity. The mobilities of the H- and L-chains of y-globulin are indicated. (b) Electrophoresis in a 10% SDS/polyacrylamide-gel of that fraction of a 1% Triton X-1000 extract of radioiodinated spermatozoa from the cauda epididymis which was eluted specifically from concanavalin A-agarose using 0.5 M-a-methylmannoside. The mobilities of the H- and L-chains of y-globulin are indicated.

**Fig. 1.** Electrophoretic analysis of the proteins extracted using Triton X-100 from the plasma membranes of mouse spermatozoa

(a) Equal numbers of spermatozoa isolated from the caput ( ▲ ▲ ), corpus ( ■ ■ ) and cauda ( ● ● ) epididymis were radioiodinated, extracted with Triton X-100 and fractionated by electrophoresis on an 11% SDS/polyacrylamide-gel. The electropherograms were cut into 1 mm sections and counted for radioactivity. The mobilities of the H- and L-chains of y-globulin are indicated. (b) Electrophoresis in a 10% SDS/polyacrylamide-gel of that fraction of a 1% Triton X-1000 extract of radioiodinated spermatozoa from the cauda epididymis which was eluted specifically from concanavalin A-agarose using 0.5 M-a-methylmannoside. The mobilities of the H- and L-chains of y-globulin are indicated.

**Abbreviation used:** SDS, sodium dodecyl sulphate.

**Immunofluorescence studies on spermatozoa from the mouse epididymis**

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Spermatozoa undergo functional maturation during their passage through the epididymis. Work on several animal species (Lea et al., 1978; Olson & Hamilton, 1978; Hoskins et al, 1979; Kohane et al., 1979, 1980; Faye et al., 1980; Moore, 1980; Voglmayr et al., 1980; Wong et al., 1981; Wong & Tsang, 1982) suggests that spermatozoa in the epididymis become coated with specific proteins which are secreted by distinct regions of the epididymis. Studies in this laboratory on the comparative protein compositions of epididymal fluid and extracts of the plasma membranes of mouse spermatozoa have shown that the corpus and cauda epididymis secrete a glycoprotein of relative molecular mass 27000 and this protein was detectable on the surface of cauda but not caput spermatozoa. In the present study, antibodies against mouse unfractionated cauda epididymal spermatozoa but not detectable in either caput or corpus spermatozoa plasma membrane (Fig. 1a). The extract from cauda spermatozoa was separated by electrophoresis into 30 distinct protein fractions. The two proteins of relative molecular masses 34000 and 44000 bound to the column when the extract of cauda membrane was applied to concanavalin A-agarose and were desorbed from it by 0.5 M-a-methylmannoside (Fig. 1b).

of the first injection. The blood was allowed to clot overnight at 4°C and then centrifuged at 750g for 15 min. The serum was heated at 56°C for 30 min and the complement-free serum was then mixed successively with erythrocytes, liver and brain cells from adult mouse, essentially according to the method of Herr and Eddy, 1980. Following immunoadsorption the rabbit antiserum was free from antibody activity against membrane and cytosolic extracts of mouse erythrocytes, liver and brain cells and there was no detectable immune reaction with luminal fluid or spermatozoan membrane extracts from the rat cauda epididymis when assayed by Ouchterlony double-diffusion in 1% agarose gels. The immunoglobulin fraction of the rabbit antiserum was purified by precipitation with ammonium sulphate and chromatography on DEAE-Sephadex A50 (Harboe & Ingild, 1973). The rabbit antibody fraction reacted strongly against luminal fluid from the mouse cauda epididymis, but there was no detectable immune reaction against luminal fluid from the caudal epididymis.

The antigen in cauda luminal fluid was identified by labelling radioactively the proteins using isethionyl[1-14C]acetimidate and immunoprecipitating the antigen with the rabbit antibody and Staphylococcus aureus Cowan Strain I. The immune precipitate was dissociated in 2% SDS and 5% 2-mercaptoethanol, and subjected to electrophoresis in SDS/polyacrylamide-gels. The electropherograms were examined by fluorography and the antigen was identified as a protein of relative molecular mass 27000. The antigen has been located in the epithelial cells surrounding the cauda epididymal lumen and coating the microvilli and spermatozoa (Fig. 1).

**Cytosolic and mitochondrial fumarases**

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In cases well characterized to date, cytosolic and mitochondrial isoenzymes are products of different genes. Two situations may be distinguished. The isoenzymes may be coded by genes with no apparent evolutionary relationship, as exemplified by superoxide dismutase (Muno et al., 1981), or more commonly by genes which have evolved from a common ancestor, as is the case for aspartate aminotransferase (Barra et al., 1980).

Fumarases seems to be different. Edwards & Hopkinson (1979) provided genetic evidence that the cytosolic and mitochondrial isoenzymes are the products of a single gene, but nevertheless the two isoenzymes are electrophoretically distinct. We decided to investigate the structural relationships between them.

Fumarases were purified from total homogenates of pig liver by fractionation with (NH₄)₂SO₄ (Kanarek & Hill, 1964) and affinity chromatography with pyromellitic acid as the immobilized ligand (Beeckmans & Kanarek, 1977).