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MUSCLE MORPHOLOGY IN NORMAL AND AT RISK FETUSES

Fetus (sex and gestation in weeks)*	Mean muscle fibre diameter (µm)	Opaque-fibres (%)	Calcium positive fibres (%)
Normal (n = 5; M; 19±1.2)	7.88 ±0.94	0.11 ±0.03	0.26 ±0.08
(A) at risk (M; 20)	12.8	3.5	3.8
(B) at risk* (M; 19)	13.2	4.3	4.8

*Termination by prostaglandins; others by hysterotomy.

don-to-tendon) biceps and quadriceps femoris muscles from all cases were removed immediately and directly frozen in isopentane cooled with liquid nitrogen. Our histological and histochemical techniques are described elsewhere; also on frozen sections we did histochemical tests for calcium (glyoxal-bis-2-hydroxyanil and alizarin-red S).¹

In the control fetuses we did not find structural abnormalities in the muscle fibres (table); there were no opaque fibres except for scattered fibres at the edge of the sections. These fibres were the only ones that displayed calcium-positive staining in this group. However, in two at risk fetuses a significant percentage of dark opaque fibres (3.5–4.3%) has been found. These fibres were intensely positive with both histochemical reactions for calcium (figure B). Further, some apparently normal fibres also displayed an increase of calcium content. Dark fibres were usually larger than normal and scattered among normal fibres; however, 5–15 dark fibres were occasionally clustered in groups (figure A). The size of individual fibres arranged in clusters was not uniform and some of

Serial frozen sections of biceps brachii from at risk fetus (A)
(A) Modified trichrome. Dark opaque fibres isolated (arrow head) or clustered in small group. (× about 200).
(B) Alizarin-red S. Dark fibres intensively positive with this reaction. (× about 280).

these fibres had centrally located nuclei. Our findings accord with other observations from fetuses^{2,4,5} at risk for Duchenne muscular dystrophy.

In this report, as well as in that by Emery and Burt,² the percentage of calcium-positive fibres is almost the same as that of opaque fibres and both are significantly high only in fetuses at risk. Hyper-eosinophilic and calcium-positive fibres might represent artifacts resulting from hypercontraction induced by injury during muscle dissection.¹ However, we carefully took away the whole muscle and in our counting of positive elements we excluded fibres at the periphery of sections. Moreover, we did not find any difference between pregnancies terminated by hysterotomy and the one terminated by prostaglandin not mentioned above. Artifacts seem unlikely.

By analogy with similar findings in cases of Duchenne muscular dystrophy^{1,6,7} we conclude that our data support the hypothesis that muscle fibre degeneration is present at an early stage of life in Duchenne muscular dystrophy, and this strongly encourages efforts directed at a prenatal test for affected fetuses.

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First Department of Obstetrics and Gynaecology,
University of Milan,
Milan, Italy

Neuromuscular Disease Service,
Istituto Neurologico "C. Besta",
Milan, Italy

B. BRAMBATI
F. CORNELIO
F. DWORZAK
I. DONES

β←-LACTAMASE-PRODUCING ANAEROBES

SIR,—We read with interest the suggestion by Dr Ingham and colleagues (Oct. 4, p. 748) that β-lactamase producing anaerobes may be responsible for the failure of penicillin therapy to eradicate *Streptococcus pyogenes* from children's throats. In a recent study of the anaerobic flora of the tonsils (to be published), we observed that the most prevalent anaerobic species (*Bacteroides melaninogenicus*) was frequently resistant on disc testing to 1 unit benzylpenicillin. The minimum inhibitory concentrations of penicillin and ampicillin were determined for 38 of these tonsillar isolates (table). Research into the drug histories of many of these children showed an alarming frequency of prescriptions for penicillin derivatives; this probably explains the high percentage of strains resistant to penicillin. Further studies are in progress to assess the penicillinase production by these oral strains.

In view of the heavy anaerobic flora to be found in the tonsillar crypts of children with recurrent tonsillitis, it follows that therapy directed at reducing the anaerobic bacterial component may be a pre-

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MIC VALUES FOR TONSILLAR ISOLATES

Cumulative % strains inhibited by					
Conc. (µg/ml)	Pen.	Amp.	Conc. (µg/ml)	Pen.	Amp.
0.007	3	..	1	32	37
0.015	11	8	2	37	42
0.03	18	13	4	53	50
0.06	21	21	8	74	58
0.125	..	..	16	97	68
0.25	..	26	32	..	82
0.5	24	32	>32	100	100

requisite for successful eradication of pyogenic streptococci with penicillin. We have shown that orally administered metronidazole achieves a statistically significant reduction in the anaerobic tonsillar flora. Controlled studies are in progress to determine the therapeutic efficacy of metronidazole in tonsillitis.

Department of Microbiology
and Public Health Laboratory,
Luton and Dunstable Hospital,
Luton, Bedfordshire LU4 0DZ

SHEENA REILLY
A. TREVOR WILLIS

ARE CORONAVIRUS-LIKE PARTICLES SEEN IN DIARRHOEA STOOLS REALLY VIRUSES?

SIR,—Workers in Britain,¹ India,² and Australia³ have noted "coronavirus-like particles" in the stools of children with diarrhoea and also in presumably normal individuals. The particles carry a regular array of projections on their surface, as coronaviruses do, but the size and shape of the particles is highly pleomorphic. There are a number of distinct types of particle, the morphology of which will be reviewed elsewhere.⁴

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Fig. 1—Electronmicrograph of coronavirus-like particles, as seen in negative staining (A). Also visible is part of large sheet of membrane (B). ($\times 144\ 000$.)

We have investigated a case of diarrhoea in a 2½-year-old Asian child who had visited Pakistan four months previously. *Campylobacter* and cysts of *Entamoeba histolytica* were isolated from the stool, and the child was treated with two courses of metronidazole. Urological investigations were negative. The diarrhoea remitted and the child was discharged without follow-up.

Examination of the differentially centrifuged ultracentrifugation pellet of the patient's stool by electronmicroscopy with negative staining revealed large numbers of "coronavirus-like particles" type 3⁴ (fig. 1). The size and shape of the particles varied greatly; however, there was a regular array of projections around the periphery of the particles, approximately 23 nm in length. In close relationship to these particles were broad sheets of membranous material, without distinctive morphology.

An analogous pellet was fixed in buffered glutaraldehyde and osmic acid and embedded in Spurr resin, and stained sections were examined. The material of the pellet consisted essentially of two components. One was a number of large, almost empty envelopes 1.3–2.5 μm long and 0.5–1.5 μm wide, and whose wall consisted of a double layer of repeating subunits (fig. 2). The subunits had a repeating pattern of 15.4

Fig. 2—Partly empty envelope with wall composed of regularly repeating subunits, seen tangentially and cross-section. Vesicular material is seen around periphery of envelope, and also some inside its cavity. ($\times 70\ 000$.)