

when in contact with tolerant host tissue,^{8,11-14} it seems that the osteogenic potency resides in the cartilage^{8,12,13} rather than in the implanted matrix.

The observation that osteoclasts make a later appearance than osteoblasts (day 10 as against day 8) is consistent with reports on osteoclast recruitment and maturation: osteoclast progenitor cells respond chemotactically to collagen and osteocalcin (gla protein) synthesized by the osteoblasts,¹⁵ and osteoblasts are then required for osteoclast formation from progenitor cells.¹⁶

In conclusion, I propose the following hypothesis to account for the events occurring in endochondral bone formation, based on the results of this study and other work discussed above: Experimental endochondral bone formation represents a series of instructive events¹⁷; the matrix implant induces fibroblasts to become chondroblasts¹⁻⁵; at maturation the cartilage produces an osteogenic factor^{8,12,13} which activates preosteoblasts to form bone locally; and osteoclasts are attracted or develop in response to the osteoblasts and their newly-synthesized products.^{15,16}

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Parasitological Society of Southern Africa

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Parasitologiese Vereniging van Suidelike Afrika

Die uittreksels van referate wat tydens die Jaarlikse Wetenskaplike Vergadering van die Vereniging, wat op 4 en 5 Julie 1985, by die Mediese Skool van die Universiteit van Natal in Durban gehou is.

Experimental schistosomiasis: concomitant immunity studies in *Praomys (Mastomys) coucha*

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The concomitant Immunity Model has been widely applied in studies of immunity to schistosomes in experimental animals. This approach involves a primary infection of the animals with schistosomes, followed at specific predetermined intervals by challenge infection with either the same or a different schistosome species. Using this method the development of substantial levels of resistance to challenge infection has repeatedly been demonstrated in various animal models, in particular the mouse.

During the present study the antelope schistosomes, *Schistosoma margrebowie* and *S. leiperi*, were investigated for their ability to induce resistance to challenge infection with *S. mattheei* in *Praomys (Mastomys) coucha*, an indigenous rodent widely used in this country as a host for schistosomes. Despite the use of different primary infection dosages and a wide range of pre-challenge intervals, no resistance could be demonstrated. In order to assess whether this could be attributed to an inability of the antelope schistosomes to induce resistance, or of the animal

model to mount an adequate immune response, a further study was carried out using a Puerto Rican strain of *S. mansoni* for both the primary and challenge infections. Although this schistosome is known to evoke strong resistance in the murine model, no such response could be demonstrated in *P. (M.) coucha*, strongly suggesting that the immune response of this host to schistosomes differs markedly from that of the mouse.

The ability of the antelope schistosomes to induce resistance to challenge infections is currently being investigated in BALB/c mice.

The pre-patent period of *Schistosoma mansoni* in BALB/c mice; a comparison of South African and Puerto Rican strains

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Mansonian schistosomiasis in Southern Africa causes considerably less morbidity in man when compared with that found in New World countries such as Puerto Rico and Brazil. As part of a study to assess the extent to which *Schistosoma mansoni* from two geographic localities [South

Africa (SA) and Puerto Rico (PR)] differ from one another, we have investigated their development and behaviour during the early phase of infection in BALB/c mice. Using an established laboratory isolate of each *S. mansoni* 'strain', male mice were infected percutaneously with approximately 32 cercariae each and studied over a 5 to 12 week post-infection period.

The infection characteristics of the two schistosomes were found to differ markedly in this definitive host, in particular with regard to worms recovered, rate of maturation, the onset of egg-laying and distribution and density of eggs in the tissues and faeces. The worm recovery of the PR strain (39%) was approximately double that of the SA strain (21%). Furthermore, the PR strain reached maturity considerably earlier and was far more productive, as measured in terms of tissue egg burdens, than the SA strain. In addition, while eggs were present in roughly equal numbers in the livers and the GITs of animals infected with the PR strain, by far the majority of eggs in the case of the SA strain accumulated in the liver.

The results of the present study suggest that the relatively mild clinical manifestations of mansonian schistosomiasis observed in Southern Africa (compared with that in Central and South America) may be attributed in part to intrinsic differences in the parasites themselves. However, further comparative studies in an alternative experimental host will have to be carried out to confirm this.

Morbidity from urinary schistosomiasis in relation to intensity of infection in Natal

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In 510 schoolchildren living in an *S. haematobium*-endemic area, morbidity from urinary schistosomiasis was assessed on clinical, radiological, histological and biochemical evidence. The results were viewed against the background of the prevalence and intensity of infection in the subjects. Clinical morbidity correlated well with the intensity of infection, the latter in turn being influenced by factors such as water contact pattern, sex and water source.

A surprisingly high prevalence (42%) of abnormalities was observed in the urinary tract of apparently normal subjects, but no relationship between the intensity of infection and structural damage to the urinary tract could be demonstrated. Urographic changes were more severe in the 11–15 year age group than in the 6–10 year group. Despite the presence of severe urographic abnormalities, no evidence of renal impairment was detected. Significant rectal involvement (76%) in *S. haematobium*-infected subjects was regarded as a reflection of the heavy worm burdens borne by these children.

The morbidity described in this study indicates a definite degree of pathology in the infected children but the impression was that they suffered only mild disability. However, given the structural lesions seen on urography and the limited sensitivity of the biochemical tests used for the assessment of renal function, definite renal pathology cannot be ruled out. Further studies on the renal status of these subjects are essential.

Faecal excretion of *Cryptosporidium* oocysts in hospitalised black children

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The results of recent studies in other parts of the world have suggested an association between *Cryptosporidium* and diarrhoeal disease, in patients without apparent immune deficiency. The highest prevalences have been reported in children. During the period January to March 1985, 259 African children, admitted to King Edward VIII Hospital, Durban, with diarrhoea, were screened for excretion of *Cryptosporidium* oocysts by microscopic examination of air-dried faecal smears, stained by modified Ziehl-Neelsen technique. All specimens were also examined for recognised potential enteric pathogens.

Cryptosporidium oocysts were detected in 11.9% of the diarrhoeic children, while none were found in the stools of 103 in-patient children without diarrhoea (controls). All children excreting *Cryptosporidium* were under two years of age, giving a prevalence of 15% for this group. Recognised potential enteric pathogens were detected in 38.7% of these children. *Cryptosporidium* was the second most common organism

detected in diarrhoeic stools, and the only one detected in 9.2% of diarrhoeic children aged less than 2 years.

These findings are consistent with those of other studies and indicate that *Cryptosporidium* warrants consideration as a potential pathogen in children with severe diarrhoea.

Prevalence of *Trichomonas vaginalis* in black patients attending the ante-natal clinic at King Edward VIII Hospital, Durban

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This study was conducted at the ante-natal clinic at King Edward VIII Hospital, which is a referral centre for peripheral clinics of the greater Durban area. Vaginal exudates from 50 patients were examined for the presence of *Trichomonas vaginalis* by direct wet-mount technique, staining with acridine orange and culture on modified cysteine peptone liver maltose medium (CPLM) and on Robinson's medium.

There was a 40% prevalence of trichomonas infection, with a 100% correlation between wet-mount and culture on Robinson's medium. Culture in modified CPLM medium proved less sensitive and acridine orange staining was positive in only 40% of the cases.

Continuous culture of local strains of *Plasmodium falciparum* and gametocytogenesis

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The successful long-term culture of local strains of *Plasmodium falciparum* is central to many epidemiological, immunological, biochemical and genetic studies. The candle-jar technique has been successfully used by many researchers to establish *Plasmodium falciparum* cultures, but the establishment of South African isolates ($n = 12$) by this technique has proved unsuccessful. The time survived in culture by these South African isolates ranged from 2 to 44 days, 44 days being the longest period a South African isolate had been maintained *in vitro*.

An alternative technique using tissue culture flasks gassed with a mixture of 3.0% O₂, 4.0% CO₂ and 93.0% N₂ has, however, proved successful with all isolates ($n = 6$). To date, five South African and one Malawian isolate have been maintained in continuous culture for times ranging from 110 to 160 days. Cryopreservation and starting parasitaemia (0.01–5.0%) did not seem to have an effect on the adaptation of these isolates to culture conditions.

Gametocyte production was found to be high. Maximum production of mature gametocytes between days 20 and 25 ranged from 1.0% to 6.7% with a mean of 4.2% and represented approximately 50% of total parasites.

Effect of *S. mansoni* (SA strain) on *Praomys (Mastomys) coucha*: a pathophysiological study

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Praomys (Mastomys) coucha has been employed extensively in this country for the routine maintenance of schistosome life-cycles and as an animal model. Yet no detailed reports exist on the pathophysiological effects of many of the schistosome species for which it is so commonly used. In the present study we have investigated the effect of a South African strain of *S. mansoni* in this animal. Male *P. (M.) coucha* were exposed percutaneously with approx. 40 cercariae and studied over a period of 2 to 34 weeks of infection.

Animals with this level of infection showed the following features. Firstly, there was no appreciable mortality as a result of the infection; secondly, the percentage worms recovered, determined 16 and 34 weeks after infection, was 21 and 24% respectively, with approximately equal distribution of male and female worms recovered on each occasion. A particularly surprising observation was the lack of any significant splenic enlargement in the infected animals. Ova and granulomata were first noted 10 and 12 weeks post-infection in the liver and gastrointestinal tract respec-

tively. The hepatic histological picture was that of isolated granulomata, with minimal fibrotic response which was confined to the granulomata themselves.

At 12 weeks of infection, the mean diameter of granulomata in the liver was $232 \pm 50 \mu\text{m}$. No appreciable modulation of the granulomatous response was apparent in this animal model since the mean diameters did not decrease significantly over time ($P = 0.1478$), even at 34 weeks of infection when the mean diameter of hepatic granulomata was found to be $160 \pm 30 \mu\text{m}$. Few ova and granulomata were observed in the gastrointestinal tract, which was borne out by the low level of faecal egg output during the course of the study. It would thus appear that the S.A. strain of *S. mansoni* employed in this study has a relatively mild effect on *P. (M.) coucha*.

Phenotypic differences in *Schistosoma mattheei* adults and ova from populations sympatric and allopatric to *S. haematobium*

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Schistosoma mattheei ova were collected from cattle in different localities in South Africa and populations of the parasite were established in the laboratory. The morphology of the tegument of adult male schistosomes and the shape of the eggs produced by the females of the different laboratory populations were selected as taxonomic characters and studied by means of scanning electron and light microscopy.

The results indicate that *S. mattheei* populations which are sympatric to *S. haematobium* possess *S. haematobium* characteristics. It is suggested that the gene pool of the parasite in these areas is infiltrated with *S. haematobium* genes via the *S. mattheei* \times *S. haematobium* hybrid originating from human hosts.

Human infection with *Schistosoma mattheei* in the eastern Transvaal

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A nine-year-old male pupil has only *S. mattheei*-like eggs in his urine and was otherwise free of concomitant schistosome infections. During 9 months of further investigations, egg totals have been recorded; egg-shape has been studied by means of photography. Snails (*Bulinus physopsis* sp.) have been infected: 48 with a single miracidium and 80 with three to four miracidia each; 8 *Mastomys* were exposed separately to cercariae from single infections, one to cercariae pooled from snails with single infections and 61 to cercariae pooled from snails with multiple infections. F2 generation results have produced *S. haematobium*-, *S. haematobium* \times *S. mattheei* (hybrid) and *S. mattheei*-like eggs.

Elaeophora poeli (Vryburg, 1879) (Filaridae) in wild Cape buffalo *Syncerus caffer caffer* (Sparrman) in western Uganda

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In six of eight Cape buffalo (*Syncerus caffer caffer*) that were culled on the Ishasha flats in the Kigezi district, western Uganda, lesions were found on the inner curvature of the aortic arch. These lesions varied from hard, white circular plaques about 10–15 mm in diameter to brown, wrinkled calcareous pea-sized nodules often almost completely separated from the underlying tissue. The anterior end of a female adult worm (~20 cm long) was embedded in each plaque with the rest of the worm lying free in the aortic lumen. A calcareous nodule supported a fibrous, thread-like atrophied worm which either hung free or was attached to the wall of the aorta. Adult male worms (50 mm) were entirely embedded within the plaques. The development of the lesions would seem to be that initially the parasites are surrounded by an intense inflammatory reaction. This is followed by necrosis and some calcification of the media. The media then becomes vascularised around dead microfilariae. The endothelium around the lesion becomes thicker, and focal calcification occurs when the free parts of the female worms shrink to fibrous threads.

E. poeli was first described from water buffalo and zebu of South-East Asia [Bernard & Bouche (1912). *Bull. Soc. Path. exot.* 5, 109–114] but little is known about its distribution in Africa following its discovery in buffalo in the Katanga province of Zaire [Sandgroun (1938). *Eerm. J. trop. Med.* 18, 108–115]. This focus seems to be narrowly defined since Dinnick *et al.* [(1963). *Bull. epiz. Dis. Afr.* 11, 37–44] did not find them in antelope in the same Ishasha region and we found a lower prevalence in the buffalo on the flats along the Lake Albert shoreline to the north-west, whereas in the similar fire-climax parkland of the Karuma falls area of the Murchison Park farther to the east no parasites were found in over 100 buffalo examined in a culling programme.

The mode of transmission is unknown but is almost certainly through a blood-sucking arthropod, but whether a dipterous fly or a tick (very heavy infestations of *Amblyoma* were common) is involved is a matter for conjecture.

Penetration of mammalian skin by *Trichobilharzia* (*Schistosomatidae*) cercariae

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The penetration of mammalian skin by *Trichobilharzia* sp. cercariae was studied in BALB/c mice over a period of 216 hours post-exposure. An estimated 85.2% of cercariae attempted to penetrate or actually did so. After 46 hours some 45.9% were found in the epidermis and 13.7% in the dermis and subcutaneous tissue. Cercariae were able to reach the vascularized deeper layers within 1 to 3 hours. Evidence that some had reached the lungs was not convincing.

An initial inflammatory response consisting mostly of neutrophils around the invading cercariae changed to a chronic one of lymphocytes and histiocytes by 28 hours. By 46 hours this had reverted to a neutrophil-dominated response. Cercariae were not recognizable as such in sections after 73 hours and multi-nucleate giant cells were present at 120 hours. Changes in the infiltrating cell population were reflected in differential counts of peripheral blood.

Permanent kikuyu pastures perpetuate nematode parasites of sheep

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Trichostrongylus colubriformis, *Ostertagia* spp. and *Trichostrongylus axei* were dominant, *Dictyocaulus filaria*, *Haemonchus contortus*, *Nematodirus spathiger*, *Oesophagostomum venulosum*, and *Trichuris skrjabini* present in moderate numbers and *Chabertia ovina* rarely present (15 of 99 necropsies) in Mutton Merinos grazing on permanent kikuyu pastures at Elsenberg Agricultural College (near Stellenbosch) in the winter rainfall area. Heavy rains in spring 1982 (101.7 mm) and summer 1983 (103.6 mm) led to deaths, due mainly to *T. colubriformis* (mean worm burdens 56 666; March 1983), which fell dramatically the following year to insignificant numbers (mean 151) under the influence of a dry spring in 1983 (37.7 mm) and summer (41.6 mm rain).

Trichostrongylus spp. and *Ostertagia* spp. are winter rainfall parasites and their presence in massive numbers in the summer of 1983 was a combination of permanent kikuyu pastures and good rains.

Does cross-infection of nematodes occur between the wild and domestic equid?

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Cross-infestation of nematodes between wild and domestic equids has not previously been studied. Sixty-nine zebras (Burchell's and Hartmann's mountain) and 24 horses from different habitats in Southern Africa were examined for endoparasites and the total nematode burdens determined. Seven nematode families were represented by 17 genera and 40 species. Some species of all the nematode families were found in all three equids, while other nematode species appeared to be restricted to the horse or to a zebra species. At Etosha, for example, where the two zebras share the same habitat, cross-infestation appeared to occur. Nematodes which

infested only one host species in other study areas, infested both zebras in Etosha. These included the following Cyathostominae: *Cyathostomum alveatum*, *Cyathostomum montgomeryi*, *Cylicocyclyus triramusus*, *Cylicostephanus bidentatus*, *Cylicostephanus calicatus*, *Cylicostephanus minutus*; in the Strongylinae, a new species of *Triodontophorus* and in the Habronematidae, *Draschia megastoma*.

To determine further whether cross-infestation occurs, Shetland foals were infested with infective larvae of large and small strongyles (cyathostomes) cultured from zebra ingesta. Preliminary studies reveal that some strongyle species recovered from the donor zebras were also recovered from the Shetland foal and include: *Cylicostephanus calicatus*, *Cylicostephanus minutus*, *Poteriostomum ratzii* and *Triodontophorus serratus*. However, these strongyle species restricted to the zebra have not been recovered from the Shetland.

Coccidia of aquatic and terrestrial hosts: sequences in co-evolution or examples of adaptive specialisation

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Piscine coccidia maintain a consistent 'eimerian' pattern of four sporocysts, each with two sporozoites. On the other hand, they grossly diverge in sporocyst structure, which is also the basis for generic division. However, this divergence appears to be independent of other differentiating features such as intestinal versus visceral sites of development, intracellular versus epicellular forms and monoxenous versus heteroxenous life-cycles. Divergence among piscine coccidia therefore assumes a mosaic taxonomic pattern. Altogether piscine coccidia appear to bear only a remote relationship to avian and mammalian host coccidia. In reptilian hosts, alongside representatives of coccidians common to mammalian and avian hosts, occur a wide variety of forms unique to reptiles. Such coccidia are either ranging from 1 to 8 to 16 and 4 (in *Caryospora* and *Pytonella* respectively), or in developing epicellularly in a fashion found among piscine coccidia, 'epieimerians' and others. Among these, one group, characteristically parasites of the gall bladder, have bivalved sporocysts found among piscine coccidia of the genus *Goussia* (as well as a typical oblong oocyst). With a background of apparent affinities between piscine and reptilian coccidia there is one fundamental difference: soft wall oocyst in fishes vs. hard wall oocyst in all reptiles. These differences in the consistency of the oocyst wall involves different processes of wall formation. In many piscine coccidia, wall-forming bodies are absent, or if present do not participate in wall formation. In *Goussia cichlidarum*, an epicellular parasite in the swimbladder of cichlid fishes, organelles analogous to wall-forming bodies appear to contribute material to an aberrant formation of the oocyst wall as well as to the hard sporocyst wall.

Diplozoon spp. (Normann, 1832) Monogenea: polyopisthocotylea on the gills of freshwater fish in South Africa

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Parasites from the genus *Diplozoon* are ectoparasites on the gills of freshwater fish and 37 species are currently described by various authors throughout the world. This genus, together with five other genera, falls under the order Diploidea of the subclass Polyopisthocotylea. In South Africa no information exists on *Diplozoon*, although a parasite from one of the other genera, *Neodiplozoon* (Tripathi, 1969), was described by Paperna in 1979. This genus, however, differs distinctly from *Diplozoon* in that there are eight to ten pairs of clamps on the opisthaptor, while the latter has only four pairs.

During the normal development of *Diplozoon*, two young individuals fuse together at the middle of the body to form a permanent cross-like figure, whereupon each individual as it is differentiates into two portions, a foliate anterior portion containing vitellaria and the bulk of the intestine, and a posterior portion containing the genital complex and the holdfast apparatus. Eggs are normally deposited during summer and hatch approximately ten days later. The first larval stage, the oncomiracidium, possesses only two clamps, which are used to attach itself onto the gill

filaments of the host. It then develops into the next larval stage, the diporpa, which cannot develop any further until encountering another diporpa.

Parasites are collected by catching freshwater fish with gill nets and by removing the parasites from the gills using a stereo-microscope. Collections were made in the eastern Caprivi, and in the Nzelele, Loskop and Vaal dams. Three *Diplozoon* and *Neodiplozoon* species were collected. All four species seem to be host specific in that only *Barbus* spp. (i.e. *B. maraquensis*, *B. trimaculatis* and *B. kimberleyensis*) and *Labeo* spp. (i.e. *L. cylindricus*) were infected. Observations on *B. holubi* (2), *B. matozae* (1) and *L. capensis* (7), *L. umbratus* (1) and *L. rosae* (5) proved unsuccessful up to now. Other fish species that have been dissected were *Dreochromis mosambicus* (25) and *Clarias gariepinus* (2), which, so far, also seem to be unaffected by these parasites.

The opportunistic nature of *Argulus japonicus* in maintaining host-parasite relationships

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Many *Argulus* species of the Brachiurian family Argulidae are known for their wide zoogeographical distribution as well as for their wide variety of fish hosts. This distinct opportunistic nature is apparent in the introduced species *Argulus japonicus* found in some freshwater localities in the Transvaal. Its opportunistic nature of host infestation stems from its reproductive strategy coupled with its highly diverse mobility. *Argulus japonicus* moves on and off its host at will, spending as long as 3 to 4 days in a free-swimming mode. It moves freely on the host itself, concentrating mainly on and in the immediate vicinity of the host's fins.

Leaving the fish occurs mainly for reproductive purposes, i.e. males looking for females (the sex ratio ranges between 1:1 and 1:10 females to males the year round in Bloemhof and Rooodeplaat dams) and females leaving the host to lay eggs on solid substrates. All females over 3 mm in length are gravid (total size range is 0.5–6 mm) and all through the year gravid females were present in samples. Percentage of hatching is 50–100% in temperatures ranging from 20–30°C, while development time is 30–12 days respectively. The reproductive data imply that *Argulus japonicus* might prefer a certain species of fish for infestation but as a result of its movements on and off the fish and the constant supply of young specimens the year round it must and does attach itself to any species of fish in the vicinity.

Host specificity of ectoparasitic trichodinids (Ciliophora: Peritricha) in South Africa and Israel

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Trichodinid ectoparasites (Ciliophora: Peritricha) can be found on a wide range of fish species. In a comprehensive study on the host specificity of these parasites found on fish in South Africa and Israel, four distinct groups can be distinguished: species found exclusively on the skin of fish like *Trichodina acuta* Lom, 1961; species found mainly on the skin, but also on the gills, like *T. heterodontata* Duncan, 1977; species found mainly on the gills, but also on the skin, like *T. mutabilis* Kazubski and Migala, 1968; and lastly species found exclusively on the gills like all the species belonging to the genera *Trichodinella* Sramek-Husek, 1953 and *Tripartitiella* Lom, 1959. The degree of host specificity was found to increase from the skin parasites towards those found only on the gills.

Use of silver impregnation methods in the study of ciliophoran fish parasites

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The sessile peritrichs (Ciliophora: Peritricha) are highly contractile organisms with few rigid structures. In order to study these organisms taxonomically, many different techniques were used. Satisfactory results were obtained by impregnation with silver nitrate and protargol, especially in revealing aspects of the infraciliature of these fish parasites.