Progress report

Intestinal protozoa

The pathogenic species of protozoa that infect the human gut are now receiving greater attention from both parasitologists and gastroenterologists. Clinical awareness of these infections is growing and there have been several diagnostic and therapeutic advances. Progress on the biomedical side has also been considerable and some interesting new techniques have been applied. The host-parasite relationship is delicately balanced in these infections and small changes in the host’s immunological responsiveness may affect the clinical outcome.

The purpose of this paper is to assemble some of the information in this field that has been published since 1973, in the hope that further interest will be stimulated. Three previous reviews\textsuperscript{1,2,3} have covered earlier publications in a similar way.

Amoebiasis

By way of introduction several more general works should be mentioned. A book edited by Jeon\textsuperscript{4} deals with the large free-living amoebae but also covers many aspects of general biological interest to the parasitologist. The pathogenic mechanisms and ecology of \textit{Entamoeba histolytica} and free-living pathogenic soil amoebae (\textit{Naegleria}, etc.) are compared in a monograph by Singh\textsuperscript{5}. A new multi-author book edited by Padilla and Padilla\textsuperscript{6} covers various aspects of the parasitology of \textit{E. histolytica} and its clinical effects, with special reference to work in Venezuela.

Amoebiasis is of great importance in Mexico from the point of view of public health and a great deal of exciting clinical and laboratory work is being done in that country. Anyone seriously contemplating further studies in this subject must consult the supplements to \textit{Archivos de Investigacion} (Mexico) where the proceedings of the annual Amoebiasis Seminars, held in Mexico City since 1969, have been published. In 1975, an international centenary conference was held in Mexico to celebrate the original discovery of \textit{E. histolytica} by Lösch. The conference proceedings have been published as a book edited by Sepulveda and Diamond\textsuperscript{7} and Lösch’s original publication has now been reprinted in English\textsuperscript{8}.

Pathogenesis

The mechanism of host tissue destruction has been further clarified by studies of the ultrastructure of the rectal tissue from dysentery patients\textsuperscript{9} and the caecal mucosa of infected guinea-pigs\textsuperscript{10,11}. Amoebae cause contact-dependent lysis of epithelial cells, and are chemotactic to neutrophils that are themselves killed on contact. Neutrophil degranulation probably contributes to the local tissue damage that includes endothelial damage leading to thrombosis of
capillaries and venules in the lamina propria. Using an isotope-labelled cell monolayer as an \textit{in vitro} substrate, cell damage was proportional to duration of contact and to the number of amoebae; extracts of amoebae caused no damage\textsuperscript{15}. The ultrastructure of amoeba-cell contact in this system suggests that membrane damage precedes lysis\textsuperscript{13}. Amoebic virulence \textit{in vitro} can also be assayed using guinea-pig leucocytes\textsuperscript{14}. The fine structure of \textit{E. histolytica} has been further elucidated with the elegant freeze-etching technique\textsuperscript{15}; recent electron micrographs\textsuperscript{16} have shown paranuclear bodies but no evidence of trigger lysosomes; it appears that membrane-bound cytolytic enzymes are released from dendritic plasmalemmal processes\textsuperscript{17}. Diamond and Mattern\textsuperscript{18} have reviewed the exciting new work on the viruses of \textit{E. histolytica}. All the axenic strains studied carried cytoplasmic icosahedral virus, and some also had a filamentous intranuclear virus or beaded intranuclear virus-like particles. All these agents lyse susceptible amoebic hosts, but the relevance of these viruses to amoebic virulence is uncertain. Quite different structures, resembling rhabdo-viruses, have been seen in the electron micrographs of several strains including the low temperature \textit{Laredo} and also \textit{E. invadens}\textsuperscript{19}.

**Strain Characterisation**

Strain virulence in man has again been correlated with virulence and infection rate in the weanling rat caecum\textsuperscript{20}; and there appears to be a correlation between strain virulence, ease of cultivation, and growth rate\textsuperscript{21}. Amoeba cultivated without bacteria do not infect the rodent caecum\textsuperscript{22}, but large inocula of crithidia-associated\textsuperscript{23} or axenic strains\textsuperscript{24} will infect and produce lesions in the hamster liver. New-born mice or hamsters can be infected with axenic \textit{E. histolytica} by direct intracerebral\textsuperscript{25} or intrahepatic\textsuperscript{26} infection, and these techniques promise to be useful in chemotherapeutic and strain comparison studies. The ease with which a strain may be axenised appears to be related to its virulence in the hamster liver, and this may decline rapidly after axenisation\textsuperscript{14}. Efficient methods of strain cryopreservation have been described\textsuperscript{27}; at 4°C trophozoites may survive for up to 12 days and cysts for up to nine weeks\textsuperscript{28}. Selective agglutination of pathogenic strains by concanavalin A has been reported\textsuperscript{29,30}, and deserves further study.

**Host Susceptibility**

Several workers have studied host susceptibility in experimental systems. Protein deficiency predisposes to caecal ulceration\textsuperscript{31}; and carbohydrate supplementation of the protein-deficient diet favours infection but reduces tissue invasion. Mice infected with \textit{Schistosoma mansonii}\textsuperscript{32} or \textit{Trichuris muris}\textsuperscript{33} are more susceptible to amoebic tissue invasion, possibly because of local tissue damage or immunosuppression. Hamster livers damaged by the intraportal injection of glass particles or ligation of a lobar branch of the portal vein, are more likely to develop amoebic abscesses\textsuperscript{34}. Inbred rat strains differ in their susceptibility to caecal invasion\textsuperscript{35}.

Blood lymphocytes from patients with invasive amoebiasis will undergo blast transformation in the presence of amoebic antigen\textsuperscript{36,37}. Patients with liver abscess may show a temporary depression of lymphocyte transformation to PHA or amoebic antigen\textsuperscript{38}. There is no definite evidence of protective immunity in man, but protection has been shown in guinea pigs following injection of amoebic antigen\textsuperscript{39}. Attempts have been made to detect copro-antibodies in human faeces, and further work is needed in this field\textsuperscript{40,41}.
Autoantibodies to human colon have been demonstrated in the sera of amoebic colitis patients using haemagglutination and gel diffusion techniques. A series of 92 fatal cases of fulminant colitis documented from Senegal emphasises the diagnostic and therapeutic aspects of this dangerous condition. The gross and microscopic pathology of the liver that occurs in colitis patients has been classified and discussed. Invasive amoebiasis may complicate infections with Strongyloides stercoralis with fatal consequences, and among Malayan aborigines heavy infections with Trichuris trichiura may be complicated by amoebic dysentery. Double infections with Shigella and E. histolytica are reported but they are probably under-recognised. The significance of the finding of amoebae in duodenal aspirates remains uncertain.

**Clinical Features**

Further cases of amoeboma have been reported and also the unusual complication of multiple stricture. Amoebiasis of the skin and genitalia is being increasingly recognised and is reported from Uganda and Papua New Guinea. Homosexual venereal transmission has been documented in one patient and perianal gangrene due to amoebiasis has been described in a diabetic. Amoebiasis may secondarily infect haemorrhoidectomy wounds and produce lesions resembling rectal carcinoma.

The clinical and radiological features of abscesses of the left lobe of the liver have been described in detail, and also the dangerous complication of amoebic pericarditis. An important paper by Barbour and Juniper compares amoebic abscesses of the liver with pyogenic ones. In a study of 30 patients, the pH of amoebic pus ranged between 5.2 and 6.7; this finding may be of diagnostic value when an empyema is aspirated. Amoebic liver abscess has been described in a patient with situs inversus, and also the abnormalities in fibrinogen metabolism and fibrinolytic activity in a series of patients. Amoebic abscesses may occasionally contain Salmonella enteriditis or S. typhi. Scragg has described the diagnosis and management of hepatic amoebiasis in children, with reference to a series of 350 cases. The often difficult medical and surgical management of pleuropulmonary amoebiasis has also received attention.

**Diagnostic Methods**

The diagnostic sensitivity of faecal examinations has been discussed together with mathematical methods for the interpretation of epidemiological data. The variable and irregular cyst excretion patterns of different subjects have again been documented. Amoebae in stools or cultures may be identified as E. histolytica using immunofluorescence and specific antisera; they may also be recognised in tissue sections by the same method, and trypan blue is a useful counter stain. A stool fixative containing sodium acetate, acetic acid, and formalin (SAF) promises to be of value in clinical practice as it allows the same preserved specimen to be used for both cyst concentration and permanent staining of cysts and trophozoites. The poor sensitivity of routine histology for the recognition of amoebae is emphasised in a retrospective analysis of 11 patients from Uganda and in a detailed clinico-pathological study of eight patients in South Carolina; fresh wet preparations must always be examined. Skin tests using an axenic strain extract (histolyticin) have been further evaluated; some control subjects
may give a delayed response and it is possible that the test itself induces an immunological response.

The most promising new serological developments are counter-current immuno-electrophoresis, and the enzyme-linked immunosorbent test; the former is rapid, relatively simple to perform, and almost as sensitive as indirect haemagglutination. Counter-immuno-electrophoresis has also been applied to the detection of antigen in amoebic pus; the technique appears to be much more sensitive than the detection of amoebae in liver aspirate or biopsy. The relative merits of three serological tests have been reported by Stamm et al. Liver abscess patients do not give positive serological tests for α-fetoprotein. The relative merits of isotope scans and hepatic arteriography have been discussed and the scintigrams of 247 liver abscess patients are reported from South Africa. Ultrasound is another useful technique and its value has been compared with isotopic scanning. Hypaque given for intravenous pyelography may also delineate a liver abscess.

**TREATMENT**

Metronidazole continues to give good results in both amoebic dysentery and liver abscess. However, five patients have developed liver abscess one to three months after treatment of amoebic colitis with metronidazole. Fisher has described a patient with liver abscess who developed a second non-contiguous abscess while on metronidazole and refers to eight other treatment failures in the literature. Treatment failure and death at 48 days has been reported after multiple drug therapy for liver abscess; another patient had three episodes of hepatic amoebiasis despite multiple drug therapy, but this was perhaps due to repeated reinvasion from the gut. Griffin has pointed out that the best results with metronidazole have been from centres where therapeutic aspiration is frequently practised; a comparative trial of metronidazole with and without aspiration has not been reported. In a trial of 66 patients given either chloroquine 500 mg daily for 10 weeks or metronidazole 750 mg tds for 10 days there were three 'drug failures' and resolution times were very similar; in another trial metronidazole gave the same results as chloroquine plus emetine. Parenteral metronidazole is now available and will be valuable in cases of amoebic colitis with perforation or ileus; its use has been documented in one patient.

Tinidazole, like metronidazole, is a nitroimidazole derivative. Its pharmacology and use in trichomoniasis, giardiasis, and amoebiasis has been extensively reviewed. So far it has no documented advantage over metronidazole for amoebiasis. The results in amoebic colitis are similar; Scrapp however, has reported particularly good results in children.

The treatment of symptomless infections remains a problem, especially in the USA where some of the halogenated quinolines have been withdrawn and furamide is not available. None of the nitroimidazoles gives particularly good parasitological cure rates and side-effects may be troublesome. A trial carried out in Washington DC has further demonstrated the effectiveness of furamide. Paromomycin is a useful alternative and possibly also dichloracetamide. Powell advocated the use of furamide in all abscess patients treated with metronidazole.

The finding that nitroimidazoles have mutagenic effects on some bacteria and can also induce lymphomas and lung tumours in mice when given in large doses has raised some doubts about the wisdom of using...
these drugs for symptomless or mild amoebic infections. It is reassuring that a follow-up of the offspring of 1469 women, treated in pregnancy over a 20-year period, has shown no damage\textsuperscript{108}. The clinical use and toxicity of metronidazole in man and experimental animals has been reviewed by Roe\textsuperscript{109}; prolonged use of this drug, as in Crohn's disease, may cause chromosomal abnormalities in circulating lymphocytes\textsuperscript{110} and occasionally peripheral neuropathy\textsuperscript{111}.

**Giardiasis**

*Giardia lamblia* is the most frequently reported gut parasite in the United Kingdom\textsuperscript{112}. Transmission occurs in temperate climates and epidemics\textsuperscript{113,114} may occur among campers\textsuperscript{115}, or when domestic water supplies are contaminated\textsuperscript{116}. American visitors to the Soviet Union, especially Leningrad, are frequently infected and their symptomatology has been fully described\textsuperscript{117}. Wolfe\textsuperscript{118} has given a useful account of the clinical features of this infection.

**Pathogenesis**

Physiological studies of this protozoon have been held back by the difficulty of *in vitro* culture but Iyer and Gaitonde\textsuperscript{119}, and Meyer\textsuperscript{120} have now described their methodology. The fine structure of the encysted parasite has been described\textsuperscript{121}. Scanning electron microscopy has shown that in mice an impression of the ventral aspect of the trophozoite remains on the microvilli at the site of contact between parasite and epithelial cell\textsuperscript{122,123}. In humans it has been shown that the microvilli and fuzzy coat are damaged at these sites\textsuperscript{123,124}. A functional correlation of these observations has been the observation of reduced levels of brush border enzymes in the jejunal mucosa of patients with giardiasis\textsuperscript{125}.

Roberts-Thomson et al.\textsuperscript{126} fed cysts of *G. muris* to mice and showed that reduction in villous crypt ratio was dose dependent. Changes regressed over the next 25 days, and during this period there was spontaneous loss of the infection. The weight gain of infected mice was significantly lower than controls. The same group of workers studied mixed infections with *G. muris* and *Trichinella spiralis* in mice; the intestinal phase of *T. spiralis* was associated with reduced counts of *Giardia* trophozoites and cysts\textsuperscript{127}. Sehgal et al.\textsuperscript{128} were able to demonstrate patent infection in albino rats fed cysts of *G. lamblia*. In athymic nude mice, an increased mortality was found in animals infected with *G. muris* and the related flagellate *Hexamita*\textsuperscript{129}; treatment with anti-protozoal drugs reduced mortality. The inability of these animals to control gastrointestinal infections may have contributed to their wasting disease.

**Clinical Features**

In endemic areas children have been the main group reported to be symptomatic and to have malabsorption. Blanco Rabassa et al.\textsuperscript{130} documented malabsorption in 31 out of 50 infected children. Tewari and Tandon\textsuperscript{131} were able to show impaired absorption of fat and d-xylose in Indians of varying ages. Wright et al.\textsuperscript{132} studied 40 expatriate adult patients, most of whom had travelled in India and South East Asia, and found malabsorption of two or three test substances (fat, xylose, and Vitamin B\textsubscript{12}) in 20. The finding of impaired Vitamin B\textsubscript{12} absorption has been only occasionally
documented before\textsuperscript{138,139}. Brandborg\textsuperscript{135} has referred to the difficulties of interpreting absorption studies in parasitic disease among patients from areas where sprue or tropical enteropathy is endemic. However, in the study by Wright \textit{et al.}\textsuperscript{132} there was a relative lack of response to tetracycline for four weeks while treatment with metronidazole for three days or mepacrine for 10 days produced consistent functional and morphological improvement. Tandon \textit{et al.}\textsuperscript{136} report free bile acids in jejunal aspirate from their patients with malabsorption and an abnormal jejunal microflora comprising mainly enterobacteria. Similar changes in microflora were found by Wright \textit{et al.}\textsuperscript{132}, who, however, obtained normal \textsuperscript{14}C glycocholate breath tests in 13 of 14 patients with malabsorption.

\textbf{IMMUNOLOGICAL ASPECTS}

Eastham \textit{et al.}\textsuperscript{137} found an increase in intraepithelial lymphocytes in the jejunal mucosa of two patients. In a more extensive study\textsuperscript{138} a significant increase in these cells was found in patients with impaired absorption compared with either infected patients with normal absorption or controls. It is possible that immunologically mediated mechanisms contribute to mucosal damage.

Webster\textsuperscript{139} has reviewed the relationship between variable hypogamma-globulinaemia and giardiasis; and Fisher \textit{et al.}\textsuperscript{140} have reviewed the radiological changes on barium follow-through examinations in patients with normal and abnormal immunological status. Other immunodeficiencies, including selective IgA deficiency, do not appear to be associated with giardiasis\textsuperscript{141}; however, jejunal IgA levels were reduced when 10 symptomatic patients were compared with 10 controls\textsuperscript{142}. In tropical countries, giardiasis is common in marasmic children\textsuperscript{148} and it is possible that reduced secretory IgA favours infection. In two patients in the USA with chronic alcoholic pancreatitis, mepacrine therapy for coexistent giardiasis rapidly reversed the malabsorption and oedema\textsuperscript{144}. Among a series of 22 symptomatic infections in Sweden, five had coexistent pancreatic disease\textsuperscript{146}. A preponderance of blood group A has been found in Australian children with giardiasis\textsuperscript{146}.

Human serum antibodies to \textit{Giardia} have been demonstrated by Ridley and Ridley\textsuperscript{147}. The indirect fluorescent antibody test was positive only in those patients who had impaired intestinal absorption; cysts were used as antigen\textsuperscript{148}.

\textbf{DIAGNOSIS}

Mahmoud and Warren\textsuperscript{149} have suggested a systematised method for the diagnosis of giardiasis. Confirmation depends upon the demonstration of cysts or trophozoites in the stool or trophozoites in jejunal aspirates, impression smears, or biopsies\textsuperscript{150}. The Enterotest capsule is an efficient method of obtaining upper gastrointestinal juice without intubation\textsuperscript{151}. Cyst excretion is irregular and unpredictable\textsuperscript{152}, the frequency of positive stool examinations is not related to the presence or absence of malabsorption\textsuperscript{152}.

\textbf{TREATMENT}

In the treatment of giardiasis the choice lies between mepacrine, metronidazole, the other 5-nitroimidazoles, and furazolidine. Wolfe\textsuperscript{118} reported a 95\% cure rate with mepacrine (100 mg thrice daily for seven days) and 81\% with furazolidine. The criterion for cure was the absence of cysts from three
consecutive stool samples one month after treatment. In another study *G. lamblia* was not eradicated from six out of nine patients given mepacrine at this dosage for 10 days\(^{138}\). Using the examination of jejunal aspirate and mucosal impression smears as well as stools, metronidazole in a single daily dose of 2-0 g for three days cured 27 out of 31 patients\(^{153}\), and a second course cured two of the remaining four. Using the same regimen, Green *et al.*\(^{154}\) report that a group of 22 patients were all cured. Trials using tinidazole have been reviewed\(^{97}\).

Muller and Lindmark\(^{165}\) have shown that metronidazole is reduced inside anaerobic organisms allowing more drug to diffuse passively into the organism, so producing very high intracellular concentrations. The site of action of the reduced drug upon anaerobic metabolism has not been defined.

**Isosporiasis**

The two species, *Isospora belli*, and *I. hominis*, have for many years been classed together as the causes of human isosporiasis; a relatively uncommon condition normally recognised by the presence of infective oocysts or sporocysts in the stool. Experimental work has now shown that these parasites are quite distinct, while *I. belli* has a simple direct transmission cycle in man, *I. hominis* is now regarded as a species of *Sarcocystis* in which man is the definitive host and the cow or pig the obligate intermediate hosts. The taxonomic problems raised by recent work on this group of Sporozoa have been discussed by Frenkel\(^ {156}\).

Sporocysts of *I. hominis* appear in the human stool nine days or more after the ingestion of sarcocyst-infected meat and excretion of sporocysts may persist for 100 days or more, apparently without producing symptoms. Antibodies have been demonstrated by immunofluorescence, using *S. fusiformis* in cattle heart as antigen, among volunteers fed raw meat\(^ {157}\) and German sanatorium patients fed regularly on beefsteak tartare\(^ {158}\); there is no cross-reaction with *Toxoplasma* antigens\(^ {159}\). The infection is often overlooked but may be surprisingly common in countries such as Holland, Germany, or Kenya where incompletely cooked meat is commonly eaten. All stages of the life cycle are not yet described, however development in the intermediate hosts is probably similar to that of *S. muris* in the mouse\(^ {160}\), and development in man is likely to be similar to *Sarcocystis* spp. in cats\(^ {161}\). In *Sarcocystis*, unlike *Isospora*, there is no schizogony in the gut cycle within the definitive host, which proceeds directly to gametogony. It should be noted that man uncommonly acts as intermediate host for another species of *Sarcocystis*—namely, *S. lindemannii*—a muscle parasite that sometimes causes myositis or systemic manifestations\(^ {162}\).

*Isospora belli* is spread directly by the faeco-oral route and is usually reported from the tropics, but it also occurs in mental institutions and accidental laboratory infections continue to be reported\(^ {163}\). Repeated schizogonic cycles occur within the epithelial cells of the small bowel causing villous shortening and functional changes; gametes are also formed and soon after oocysts appear in the stool. Many infections are symptomatic and a prolonged debilitating sprue-like illness may result from this under-recognised condition\(^ {164}\); other clinical features are fever, blood eosinophilia, and stool Charcot-Leyden crystals. Treatment requires further study; however, cotrimoxazole 2 g daily for three weeks was successful in a patient in Israel\(^ {165}\).
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as was a 49-day course of pyrimethamine and sulphonamide in an American negro who was originally infected in North Africa in 1944. Nitrofurantoin is used to treat coccidiosis in domestic animals and deserves further study in man.

Balantidiasis

Pigs appear to be the main reservoir of infection and infection rates may reach 20% in New Guinea where human contact with pigs is particularly close. Infections in Moslem countries and mental institutions result from direct transmission or a rodent source. The fully documented outbreak in Micronesia followed a typhoon which contaminated surface water supplies with pig faeces; the outbreak was self-limited and multifocal, suggesting that person to person infection was unusual. The rare fatal cases may show extensive ulcerative enteritis. Concurrent Trichuris infection may favour tissue invasion. Metronidazole was used successfully to treat 20 infections in Venezuela, but it failed to cure three out of four asymptomatic infections in Micronesia. Nitrimidazine (Naxogin (R)) cured three symptomatic cases in Mexico, and 12 out of 17 children in Columbia who received 500 mg daily for five days. The ultrastructure of this parasite has now been described.

Other parasites

A rectal biopsy from a 3 year old girl, who presented in Tennessee with a five-day history of severe diarrhoea and vomiting, showed parasites resembling Cryptosporidium attached to crypt epithelial cells; they measured 2-4 nm and were demonstrated by toluidine blue staining and by electron microscopy. The illness was severe but self-limiting; a barium enema had shown spiculated ulceration of the rectosigmoid and descending colon. This parasite should be looked for again in similar situations.

The amoeboflagellate, Dientamoeba fragilis, was found in 4-2% of the preserved faecal specimens from 43 029 subjects in Canada. The disputed pathogenicity of this parasite is fully discussed in this paper; suggestive symptoms are diarrhoea or loose stools, abdominal pain, and anal pruritus. A positive association was found in this survey between D. fragilis and threadworm (Enterobius vermicularis), thus supporting the hypothesis that the egg of this worm acts as a mechanical vector for the protozoan.

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