cysts is not sufficient to determine with
certainty a species of amoeba, it is neces-
sary to study with great care all mor-
phological characters during its
evolution and, if this is not sufficient,
all its biological characters”.
Regrettably none of the eminent para-
sitologists attending that meeting gave
Emile BRUMPT’s hypothesis their full
support. Professor Warrington YORKE
in the following discussion of Emile
BRUMPT’s paper gave no positive
example of any experiments he himself
had carried out to substantiate or deny
Emile BRUMPT’s proposals as to the pre-

cence of E. histolytica and E. dispar.
YORKE’s final conclusion was “caution
should be exercised in interpreting Pro-
sessor BRUMPT’s experiments”. “For his
own part (i.e. YORKE) he preferred to
remain in this matter a unitarian”.
Dr. H. Lyndhurst DUKE endeavoured
to approach Emile BRUMPT’s hypothe-
sis by comparison with the situation
pertaining at that time with the Afri-
can trypanosomes. DUKE’s concluding
remarks were as follows - “He would
put it as a pure hypothesis and suppose
that there was only one species of amo-
eba—a polymorphic species, widely dis-
tributed, which varied in its physiological
attributes—known as E. dysenteriae. This
parasite was a parasite of man, well
adapted to him, but with the degree of
adaptation differing in different cir-
cumstances. Occasionally, this adapta-
tion to man broke down, resulting in
dysentery. He did not know whether
that appealed to those present as a sen-
sible proposition, but it seemed to him
the closest parallel he had ever met to
the line of suggestion which they had
been putting up lately in Uganda about
the trypanosomes; and other parasitic
protozoa were presumably governed by
similar factors”.

Dr. J.G. THOMPSON remarked that there
were three “specially important” points
to be considered. Firstly, transmission
from host to host thus raising virulence.
Secondly, the dose of cysts ingested.
Lastly, the bacteria flora present in the
gut. He gave no conclusion.
Dr. C.M. WENYON said that “BRUMPT’s
paper turned on two questions, E. his-
tolytica and E. dispar. With regard to
the two larger forms, Professor
BRUMPT’s view if adopted would intro-
duce clinical difficulties. On receiving a
laboratory report that four-nucleated
cysts of the histolytica type were pre-

sented in the patient’s faeces the physician
at once proceeded to inject emetine, but
with Professor BRUMPT’s theory put into
practice, when four-nucleated cysts of
the histolytica type were reported the
clinician would have first to ask “It this
E. dispar or E. histolytica?”. If E. dis-
par the patient should be left alone, if
E. histolytica he should be given eme-
tine, and the only way in which the
matter could be settled was to inject
these cysts into the rectum of a kitten,
closing the anus with collodion, and
waiting for a few days to see whether
the animal developed true dysentery or
not. That unfortunate position was
parallel to that which confronted the
bacteriologist and the clinician at the
present time with regard to the treat-
ment of cases of diphtheria. From the
practical point of view the unresolved
question, as to whether there were two
distinct species or not, did introduce
tremendous difficulties in treating cases”.

Finally, WENYON said “With regard to
the general principle of the tests, he had
always viewed with great prejudice the
pathological or pathogenic difference
tion of species. A species had always
represented to his mind a morphologi-
cal idea, but he thought a consistent
effort should be made to maintain it. One wanted to keep to the idea of a morphological species which bred true. There were certain pathogenic races, but they should not be called species, and be thought it worth while to plead that the ordinary idea of species should not be prostituted”.

Emile BRUMPT remarked after the foregoing discussion - “In conclusion, I may assert that in temperate countries there exists a high percentage of individuals harbouring four-nucleated cysts. They are not dangerous for those about, they have no need to fear liver complications, and for them there is no necessity for an intensive treatment. Nevertheless, many other experimental researches must be done. It complicates the work of clinicians and scientists, but we all know that science is difficult, and the way is long to reach the truth”.

A very important point to make is that Clifford DOBELL, an accepted leader in the field of amoebiasis was not present at the meeting, or if he was present he was not prepared to comment; was he yet another scientist not prepared to accept BRUMPT’s hypothesis?

At about the time of Emile BRUMPT’s original paper (3) i.e. raising the question of E. dysenteriae/E. dispar, many scientists were endeavouring artificially to cultivate amoeba. Among these were BARRET and SMITH (1) and notably BOECK and DRBOHLAV (2).

CLEVELAND and SANDERS (8) working with cats produced a long dissertation regarding associated bacteria and liver infection with E. histolytica. No mention or reference was given to BRUMPT’s work.

Some scientists, including HOARE (13) and ELSDON-DEW (12), published work relating to the size of cysts. This phenomenon had already been clearly stated by Emile BRUMPT in his paper read to the Royal Society of Tropical Medicine and Hygiene. But no mention is made of BRUMPT’s observations.

At a meeting of the Société de pathologie exotique at the Pasteur Institute - June 9, 1926, M. MESNIL’s answer following Professor E. BRUMPT’s presentation of anatomical specimens of Entamoeba dispar was as follows (4).

“M. MESNIL. The statistical data put forward in support of our colleague BRUMPT’s theory may be given, I think, another interpretation : the important role of the intestinal flora in the invasive amoebiasis. During the war, amoebiasis was epidemic on the occidental front as well as in Macedonia. There is no doubt, that at this occasion numerous cyst carriers were disseminated in France, England etc. If they have not been the starting point of new cases of acute amoebiasis, what would have happened in tropical countries, one may think that the reason is due to the disappearance of the intestinal flora accompanying the amoeba, which was easily transferred from one individual to another by the mere fact of promiscuity conditions during the war. The amoeba in a healthy carrier, although having an Entamoeba dispar behaviour is however an Entamoeba dysenteriae”.

In answer Emile BRUMPT ... “In my opinion, there is no action of the intestinal flora on Entamoeba dispar. If this intestinal flora was able to allow transformation of what I call Entamoeba dispar into Entamoeba dysenteriae, how to explain the extreme scarcity of acute amoebiasis cases in tropical countries such as meridional Brazil where numerous 4 nuclei cyst carriers coming from Europe are found. With regard to the relative scarcity of acute amoebiasis in France since the end of
the Great war, I think this phenomenon is easily explainable by the fact of a return to normal life and the considerable decrease of exposure to faecal elements*.

From all the foregoing, and numerous other publications, it appears quite obvious that very few scientists at that time were prepared to consider Emile BRumpt’s discovery as realistic.

To judge the height of opinion at the time of Emile Brumpt’s (3) paper, it would be fair to report that there were two main antagonists. One in France and one in England.

In France the main opponent of E. dispar was F. Mesnil from the Pasteur Institute.

Mesnil, to avoid the recognition of Emile BRumpt’s discovery suggested the important role of the associated flora, without any demonstration of his hypothesis. A battle followed and Emile Brumpt was refused access to the Academy of Science ... the reason was E. dispar ...

In England, Wenyon was against Emile BRumpt’s hypothesis and in his immense volume on “Protozoology” (24) he writes “Emile BRumpt (1925), however, suggests that there exist two types of amoeba included under the name E. histolytica, the one infective to kittens and the other not. To the latter he gives the name E. dispar, and suggests that it accounts for many of the carrier cases in countries where amoebic dysentery is uncommon. The writer does not believe that physiological data of this kind afford a means of distinguishing species”.

It is significant that Wenyon dedicated his book to MESNIL.

Masses of research work on amoebiasis followed throughout the years with almost no mention whatever of Emile BRumpt’s work, except the publication of Lucien BRumpt & Ho Thi SANG in 1961 (6) in which they assert that non-pathogenicity is a fixed and irreversible character whatever artefacts are used, these experimental notions being confirmed by the absence of haematophagia, the clinical and the epidemiology of amoebiasis. This state of affairs was suddenly stopped about 1978, more than 50 years since Emile BRumpt’s publication of 1925, when Sargeaunt produced irrefutable evidence that there were indeed two species within what was known as the species E. histolytica (21).

Sargeaunt’s work was based on the marriage of two sciences: Bacteriology and Biochemistry. Briefly, it consisted of culturing faeces containing cysts to grow out the trophozoites which were harvested and applied to thin layer starch gel electrophoresis. Using four enzyme systems it was seen that each strain of amoebae showed unique banding. Particularly among the many thousands of isolates made the banding of E. histolytica and E. dispar always gave clear and reproducible results which were directly related to disease in the host. All other parasitic amoebae found in man have also been identified and have shown typical species banding (22).

After the first few publication of SARGEAUNT’s research, a great plethora of work, using varying approaches, was undertaken by various groups of scientists, around the world, and with one exception all confirmed SARGEAUNT’s (21) original findings. The exception was Mirelman (14), who tried to show that associated bacteria in culture was the reason for the difference of banding on electrophoresis. However, Mirelman’s results were shown by Sargeaunt (19, 20) to be erroneous, and that his cultures were contaminated with more than one species of
amoeba. The publication of his results was sufficient to influence some amoebologists that he was correct. Notably among those who followed his idea was DIAMOND (9) who informed SARGEAUNT that he did not believe there were two species, (i.e. E. histolytica and E. dispar) and therefore the electrophoresis work was incorrect.

But some time later, DIAMOND, at a minor meeting in Italy (10) announced he had changed his mind and told SARGEAUNT that he now believed in two species.

Reviewing a more contemporary situation there are 55 chapters, each written by a separate scientist working in the field of amoebiasis, in the book edited by RAVID (18). Apart from the several mentions by SARGEAUNT (chapter 25) of Emile BRUMPT's work, only one other author mentions him once (OROZCO et al. chapter 21) and then only to disparage his hypothesis.

Many new approaches have been made which establish the 2 species as fact. From all the work generated, 6 main publications serve to show the immaculate differences between the 2 species (see table I.)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARGEAUNT et al.</td>
<td>Isoenzyme electrophoresis</td>
</tr>
<tr>
<td>TANNICH et al.</td>
<td>Genomic DNA/restriction enzyme fragment analysis of a single gene</td>
</tr>
<tr>
<td>PETRI et al.</td>
<td>Monoclonal antibodies to galactose-specific adherence lectin</td>
</tr>
<tr>
<td>EDMAN et al.</td>
<td>Different immuno dominant variable surface antigen</td>
</tr>
<tr>
<td>QUE &amp; REED</td>
<td>Nucleotide sequencing of small sub-unit ribosomal DNA</td>
</tr>
<tr>
<td>CLARK &amp; DIAMOND</td>
<td>Ribosomal RNA genes</td>
</tr>
</tbody>
</table>

The one piece of work to highlight would be the research undertaken by TANNICH et al. in 1989 (23), showing genomic differences between “Pathogenic and non-pathogenic E. histolytica”.

However, in 1995, in France, a synthetic study was published on E. histolytica and E. dispar pathogenic and non-pathogenic species and once again “gave rise to controversy” (15).

The proof of two species became widely accepted and in 1997, the World Health Organization (WHO) (25) issued a formal declaration to state that E. histolytica and E. dispar were a fact. However, as so often happened previously, they have forgotten to highlight the research of the two main progenitors of the two species: i.e. Emile BRUMPT and SARGEAUNT.

As stated by Emile BRUMPT 70 years ago, and again reiterated by SARGEAUNT, there is no need to treat infections with E. dispar, a fact now accepted by W.H.O in publication. Before the use of N-imidazoles to treat amoebic infections, Emetine, with all its dangerous side effects, was used as a treatment. We must ask how many subjects suffered as a result of this treatment when they were only infected with E. dispar?

In conclusion we should look at the amoebic research which is composed, apart from the very early work (pre-1900), of three parts. The first part is the work of E. BRUMPT. The third part is the work of SARGEAUNT. The second part is all the work undertaken between parts one and three. This second part contains little, apart from treatment, that is worthwhile, and consequently most can be expurgated from our minds. Every point that Emile BRUMPT postulated 70 years ago we now know was absolutely true, and consequently this is what we should teach our students.
Bibliography


Intervention en séance

J. Théodoridès :
Je souhaiterais rappeler la rivalité qui existait dans la décennie 1920 entre le laboratoire de parasitologie de l’Institut Pasteur et le laboratoire de parasitologie de la Faculté de médecine de Paris. L’opposition exprimée par F. MISNIL lors de la communication prècèdes d’E. BRUMPT sur Entamoeba dispar en est une preuve manifeste.

J.C. Petithory & F. Ardoin d’après les notes de L.C. Brumpt (†)
Qualité en parasitologie et biologie, Centre hospitalier de Gonesse, 25 rue P. de Theilley, B.P. 71, 95503 Gonesse, France.

Mots-clés : anémie, gourme des mineurs, ankylostomidé, *Necator americanus*, *Ancylostoma duodenale*, auto-infection, Lucien Brumpt

Key-words: Ankylostomidae, *Necator americanus*, *Ancylostoma duodenale*, anemia, auto-infection, Lucien Brumpt

Summary: Multiple experimental and human auto-infection with *Necator americanus*, *Ancylostoma duodenale*.

In 1997, the number of people infected with either or both of the major human hookworm species, *Ancylostoma duodenale* and *Necator americanus*, has been estimated to 1277 millions. It may be interesting to give here some data of experimental results concerning these intestinal nematodes. As early as 1940, a new technique for the treatment of polyglobulic patients was developed by infecting them with *A. duodenale*. The results obtained were excellent, the worm load was easily modulated and risks of side effects were far less important than with the use of radio-active-phosphorus treatment (leukemia). Therefore, more than 50 cases have allowed us to study, among any other aspect, the eosinophil responses to hookworm infection.

Self-induced infections (L.B.) six times with *A. duodenale*, twelve times with *N. americanus* were conducted, corresponding to natural infections or reinfections. These investigations have resulted in showing and confirming important differences between *A. duodenale* and *N. americanus*. The former being more pathogenic, showing the existence of a diapause (hypobiosis) and being congenitally transmitted.

Clinically, "la gourme des mineurs", that is a marked skin reaction, of little importance with a primary infection, becomes increasingly more important with further reinfections. The migration of larvae through the lung is clinically totally silent, but on the 4th day of infection appears what is called "le catarhne des gourmes", irritation of the superior respiratory tract: trachea, larynx, pharynx. Occasionally, at the end of the first month of infection, a quite severe duodenite can be reported.

The protective immunity to hookworm infecting human is extremely limited. After multiple successive self-infection, it was shown that the percentage of transformation of *N. americanus* larvae into adult worms, moderately decreased but never disappeared and therefore, there is little hope for a vaccine development.

On a skin protection viewpoint, it has been demonstrated that synthetic textiles, slow down or even prevent the through passage of *N. americanus* larvae.

The current treatments have proved efficacy, they are however insufficiently used in developing countries, this is probably due to economic reasons.

Résumé :

Cinquante cas d’infestations thérapeutiques par *Ancylostoma duodenale* et *Necator americanus* pratiquées à partir de 1940, pour traiter, avec d’excellents résultats, des polyglobuliques, ainsi que de nombreuses auto-infections avec ces mêmes espèces d’Ankylostomidiés (6 fois avec *A. duodenale* et 12 fois avec *N. americanus*) ont permis à Lucien Brumpt de mettre en évidence et de confirmer d’importantes différences entre ces deux nematodes, en particulier la plus grande pathogénicité d’*A. duodenale*, l’existence d’une diapause (chétivisme) pour cette espèce seulement, ainsi que sa transmission congénitale. Au plan clinique, la “gourme des mineurs”, peu marquée lors de la primo-infection, devient de plus en plus importante lors des réinfections. Si le passage des larves au niveau des
poumons est totalement silencieux, par contre, vers le 4ème jour survient "le catarrhe des gourmes", irritation des voies aériennes supérieures. La duodénite apparaît à la fin du premier mois, dure un mois, s'accompagne d'une importante diarrhée, et ne s'observe qu'avec A. duodenale. Dans l'ankylostomose comme dans la nécatorose, l'éosinophilie sanguine suit la courbe décrite par Lavié. Au plan épidémiologique, le tissu synthétique freine ou empêche le passage des larves qui restent infectantes après un court séjour dans des milieux salés, même à assez forte concentration. L'immunité acquise par l'homme vis-à-vis des Ankylostomides est faible.

**Introduction**

La plus récente estimation (1997) du nombre de personnes infestées par les Ankylostomides dans le monde est de 1 277 millions (9). Un tel chiffre montre l'importance de ce parasitisme et la nécessité d'études complémentaires pour mettre au point une stratégie de lutte efficace contre ces nématodes. Différents travaux ont été faits à partir d'auto-infections par *Ancylostoma duodenale* et *Necator americanus*. Il s'agissait habituellement d'infections uniques, non répétitives. *Beaver* (3), par exemple, s'est infesté une seule fois avec des larves de *N. americanus*, sans se traiter, ce qui lui a permis de montrer que la durée de vie de ce ver pouvait atteindre 18 ans. Celle d'*A. duodenale* est plus brève, de 5 ans en moyenne (5).

La démarche de L.C. Brumpt a été différente: il s'est infesté de manière répétitive, à intervalles de temps variables, dans des conditions qui se rapprochaient des conditions épidémiologiques habituelles. Il a également étudié expérimentalement l'ankylostomose et la nécatorose à l'occasion de plus de 50 cas de polyglobulies traitées par ces deux parasites hémato-phages. Le but de la présente note est de rappeler un certain nombre de données que ses travaux ont permis de déterminer ou de préciser. Ces données concernent: la morphologie et la biologie d'*A. duodenale* et de *N. americanus*, leur épidémiologie, l'évolution clinique, en fonction de leur cycle, des affections dont ils sont responsables, l'évolution de l'hyperéosinophilie sanguine, l'immunité qu'ils suscitent, le traitement de l'anémie qu'ils provoquent.

**Matériel et méthodes**

La méthode, relativement simple, utilisée par L.C. Brumpt pour mener à bien ses différents travaux et pratiquer ses infestations thérapeutiques ou expérimentales peut être résumée de la façon suivante.

Les souches de *N. americanus* utilisées provenaient d'Afrique tropicale et celles d'*A. duodenale* de Guyane française.

Les larves strongyloides infestantes, mobiles, obtenues par coproculture sur papier buvard, âgées de 8 à 15 jours après l'émission des selles, ont été tout d'abord lavées dans une solution à 9 pour mille de ClNa stérile. La richesse en larves de quelques gouttes de solution calibrées à 50 µl a été ensuite déterminée. Puis un nombre défini de gouttes a été déposé sur un morceau de papier buvard d'environ 10 cm² préalablement humidifié avec de l'eau du robinet et appliqué sur la peau de l'avant bras. Le papier filtre a été laissé en place pendant une demi-heure, temps nécessaire à la dessiccation progressive qui est indispensable à la pénétration des larves. En effet, si l'on fixe sur la peau un cylindre de verre contenant des larves
dans une assez grande quantité d’eau, les larves nagent sans chercher à pénétrer (5, 10).
Le nombre des œufs a été déterminé par une méthode décrite à plusieurs reprises (11, 16) et que nous rappelons brièvement:
- 5 grammes de selles sont déposés dans un petit verre à pied gradué, et progressivement dilués dans 50 ml de ClNa à 9 % à l’aide d’un agitateur en verre. La dilution habituellement utilisée est au dixième, sauf en cas de fluidité ou de dureté anormales des selles.
- à l’aide d’une pipette Pasteur calibrée, fournissant 50 gouttes par ml, une goutte de la suspension précédente permet, par examen microscopique, le dénombrement X des œufs observés.

Tableau I.

Comparaison entre *Necator americanus* et *Ancylostoma duodenale*.

<table>
<thead>
<tr>
<th>répartition géographique</th>
<th><em>Necator americanus</em></th>
<th><em>Ancylostoma duodenale</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sud de la Chine</td>
<td>Sud de l’Inde – Indonésie, Vietnam</td>
<td>Nord de la Chine</td>
</tr>
<tr>
<td>Afrique de l’Ouest, centrale et du Sud Antilles, Amérique tropicale et sud des États-Unis, sud Pacifique, Australie</td>
<td>Afrique du Nord, Côte d’Ivoire, Nigeria</td>
<td></td>
</tr>
<tr>
<td>capsule buccale de l’adulte</td>
<td>2 lames semi-circulaires</td>
<td>2 paires de dents en crochets</td>
</tr>
<tr>
<td>transmission au nouveau né</td>
<td>absente (18)</td>
<td>présente (14)</td>
</tr>
<tr>
<td>contamination par voie bucale</td>
<td>0,03 ml</td>
<td>0,15 - 0,23 ml (14)</td>
</tr>
<tr>
<td>délai d’apparition des œufs après l’infestation</td>
<td>49 à 56 jours (14, 18)</td>
<td>38 à 53 jours (5, 14)</td>
</tr>
<tr>
<td>diapause = chétivisme (arrêt du développement)</td>
<td>absente</td>
<td>présente (6)</td>
</tr>
<tr>
<td>perte quotidienne de sang due à un ver</td>
<td>5 000 – 10 000</td>
<td>10 000 – 25 000 (14)</td>
</tr>
<tr>
<td>ponction quotidienne d’une femelle</td>
<td>50 à 200 (3, 16)</td>
<td>125 à 240 (1, 3)</td>
</tr>
<tr>
<td>œufs par gramma de selle et par femelle</td>
<td>36 à 45 µm</td>
<td>36 à 45 µm</td>
</tr>
<tr>
<td>longueur moyenne :</td>
<td>40 µm</td>
<td>40 µm</td>
</tr>
<tr>
<td>longueur minimum – max :</td>
<td>64 à 76 µm</td>
<td>56 à 71 µm</td>
</tr>
<tr>
<td>longueur moyenne :</td>
<td>70 µm</td>
<td>60 µm</td>
</tr>
<tr>
<td>nombre de blastomères à l’émission</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>larves stronglyoides :</td>
<td>500-600 µm/25-30 µm</td>
<td>500-600 µm/25-30 µm</td>
</tr>
<tr>
<td>extrémité postérieure</td>
<td>bien développés</td>
<td>bien développés</td>
</tr>
<tr>
<td>styles buccaux</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sex-ratio mâle femelle</td>
<td>1,5</td>
<td>1 (14)</td>
</tr>
<tr>
<td>charge parasitaire pour 1000 œufs par gramma de selle</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>durée de vie</td>
<td>3 à 18 ans (3, 14, 18)</td>
<td>2 à 6 ans (5, 18)</td>
</tr>
<tr>
<td>pathogénicité chez l’homme</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>hémorragie intestinale</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>


Résultats

Comparaison entre
*A. duodenale* et *N. americanus*

Les résultats obtenus, complétés par les données de la littérature, figure dans le tableau I. On peut constater que les différences entre les deux espèces d’Ankylostomidé sont importantes, tant aux plans de leur morphologie que de leur biologie. Il convient donc de ne pas les