OBSERVATIONS ON *ENTAMOEBA GINGIVALIS*
FROM THE HUMAN MOUTH, WITH A NOTE
ON THE TRICHOMONAD FLAGELLATE *TE-
TRATRICHOMONAS BUCCALIS* N. SP.

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(With Plates XX—XXII.)

CONTENTS	
	PAGE
Introduction	537
Methods	538
(a) Observations on the living organism	538
(b) Fixed and stained material	539
Morphology	540
(a) The living amoeba	540
(b) The stained organism	542
(c) The food of <i>E. gingivalis</i>	543
Description of cases	548
Conclusion	553
A note on the trichomonad flagellate <i>Tetratrichomonas buccalis</i> n. sp.	554
References	557
Explanation of Plates	558

INTRODUCTION.

THE claim made for the first time by Barrett and Smith (1914 a) some two years and a half ago, that the causative agent of Pyorrhoea Alveolaris is the *Entamoeba gingivalis* led the writers to enquire into the question ; the results of which enquiry are set forth in the following paper.

From the first we were not convinced that the mere association of an organism with a particular set of circumstances in a certain percentage of cases is proof that we are dealing with cause and effect. Nor, when yielding to the inhibitory effects of some specific reagent the organism disappears, and *pari passu*, the abnormal circumstances also show a tendency to disappear, can we yet agree that the association of one with another as cause and effect has been definitely established.

So far, all attempts to reproduce the disease by inoculation of healthy tissues with discharge containing amoebae have failed. In regard to treatment, if we grant that certain cases do well with emetine after having proved refractory to all other methods, it remains still to be proved that the improvement is due to the amoebicidal action of the drug and not to some other possible effect, such (as has been suggested) as its bactericidal action or its possible beneficial effect upon the phagocytic function of the leucocytes.

It is unnecessary for us in this paper to discuss the nomenclature of *Entamoeba gingivalis* nor need we discuss the historical aspect of the question; all this has been done quite recently and very thoroughly in one or two papers dealing with this problem, *e.g.* Smith and Barrett (1915 b) and Craig (1916).

We have contented ourselves with two principal lines of attack on the problem. First, a careful investigation into the structure of the amoeba by exact cytological methods, particular attention being directed to the nature of the inclusions, and, second, an examination of a number of mouths, some healthy, others showing a variety of pathological conditions.

We have not attempted to investigate the conditions influencing the trophic existence of the amoeba, as for example, the effect of the saliva as regards the amount secreted and its chemical composition, or the influence of the fermentative changes proceeding in the buccal cavity.

METHODS.

(a) *Observation on the living organism.*

For making observations on the living amoebae we have employed the warm stage with success.

We have found it convenient to make our preparations for examination under these conditions as hanging-drops on cover-slips placed over cavity slides and sealed with wax. By employing this method it is possible to lift the cover-slip after making observations and fix off the preparation at any desired moment.

In making cover-slip preparations we have taken a small quantity of material from the mouth, whether pyorrhoea pus or food or other *débris* by means of a sterile needle or capillary pipette and have teased it up with a platinum loopful of a diluting solution.

The solutions used for this purpose have been normal salt solution, saliva, salt-citrate solution of the strength recommended by Minchin¹ for the examination of trypanosomes, and salt-citrate solution to which 15 % white of egg has been added. By the use of the last-mentioned solution it is possible to get very good film preparations on fixation, for the egg-albumen in coagulating adheres to the cover-slip and holds most of the bodies present in the film.

It is important in making hanging-drop preparations to use only the minimal quantity of diluting solution, so as to ensure a thin layer of liquid when the majority of the leucocytes and organisms will be found on the glass surface, and not on the lower surface of the drop. If too much solution is taken, not only is it very often impossible to use high powers in the examination of the organisms, for most of the latter gravitate to the lower surface of the liquid, but when the cover-slip is lifted, and dropped on to the fixative, all those bodies on the lower surface of the drop are washed off and are lost. We have found normal salt solution and salt-citrate solution with or without egg-albumen equally good as the diluting solution.

(b) *Fixed and stained material.*

As fixatives we have used Maier's solution which is very similar to Schaudinn's sublimate alcohol, Bouin's fluid, and absolute alcohol.

The last is especially useful for rapid work when methyl-green is used as the subsequent stain. Maier's and Bouin's solutions appear to give equally good results but we have used Maier's solution most frequently.

After fixing we have followed the usual procedure for the manipulation of wet films, washing out the fixative and finally bringing the preparation into the desired staining solution. It may not be out of place at this point to call attention to the utter uselessness of dry-film preparations for the exact cytological study of mouth amoebae or of any other protozoa. We have seen particulars given for the making of such dry-films by the usual rapid bacteriological methods and recommending the use of certain rapid stains such as thionin-blue.

¹ 1 gram. sodium citrate and 1 gram. sodium chloride dissolved in 200 c.c. distilled water.

Such methods may be of service in diagnosing the presence or absence of the amoebae in a given preparation but even then their use and value is questionable especially if it should happen that the material contains only small examples of *E. gingivalis*. It is absolutely essential, as Craig points out, to keep the film moist during the whole of the fixing and staining processes if any adequate idea of the structure of the organisms is to be obtained.

We have found iron-haematoxylin to be the most useful stain for revealing the detailed structure of the organisms and most of our films are stained with this.

Dobell's iron-haematein¹ is a very good stain which is especially useful on account of the rapidity with which it can be used. After washing out the fixative the preparation is treated in the mordant, 1 % ferric ammonium alum in 70 % alcohol for ten minutes. It is then rinsed in 70 % alcohol and stained for ten minutes in 1 % haematein in 70 % alcohol, following which, it is differentiated in the mordant or in acid 70 % alcohol, and finally washed in several changes of ordinary 70 % alcohol. By the use of this stain one can quickly obtain well-stained preparations presenting clear pictures of the detailed structure of the organisms.

Other stains which we have employed are Jenner's blood stain, Giemsa's stain, Unna's polychrome methylene-blue, safranin and licht-grün, Mann's methyl-blue eosin, and methyl-green. In using the last-mentioned stain we have found it best to fix the preparation in absolute alcohol for about ten minutes and then take down through graded alcohols to water, after which it is floated on a solution of methyl-green in 1 % acetic acid for a minute or so and then mounted on a slide in a small quantity of Ripart and Petit's medium and sealed round with marine glue. Since this stain has only been used to investigate the nature of the round inclusions within the amoeba and also the character of the amoeba nucleus its impermanency is a matter of no consequence.

MORPHOLOGY.

(a) *The living amoeba.*

We are not in a position to add anything new to the published accounts of the structure, appearance, and movements of the living amoeba in its ordinary vegetative stage of existence. The recent paper by Craig (1916) deals fully and adequately with these matters.

¹ Dobell, C. C. (1914). Cytological studies on three new species of Amoeba. *Arch. f. Protist.* xxxiii. 143.

When a preparation of material from the mouth is first examined it is not at all easy to pick out the amoebae especially if there are large numbers of polymorphonuclear leucocytes present. After a short time however one's eye becomes accustomed to the field and one can then pick out from the vast numbers of round bodies certain ones which have a denser appearance and a brighter green shimmer than the rest. These are the amoebae and if they are examined under the high power it will be found that they appear quite different from the polymorphonuclear leucocytes, in which it is often possible to distinguish the lobed nucleus surrounded by finely granular protoplasm.

If examined on the warm stage an amoeba will soon be seen to send out small lobose pseudopodia of clear ectoplasm in various directions in which case it will not progress much in any given direction. One large pseudopodium may be extruded and the amoeba will then take on a "limax" like appearance (Pl. XX, fig. 1) and the movement will be chiefly one of translocation.

As already mentioned the ectoplasm is hyaline in appearance in the pseudopodia, whilst the endoplasm is coarsely granular and is made up very frequently of a large number of vacuoles, some of which appear to be empty whilst others contain inclusions of various sizes and shapes.

If a preparation showing actively motile amoebae is examined for any length of time, half to three-quarter's of an hour, on the warm stage it will be observed that as an amoeba moves about, its posterior end frequently becomes entangled in clumps of bacteria and polymorphonuclear leucocytes. The majority of these adherent bodies appear to be a hindrance to its continued progress, and the organism seems to be trying to get rid of them; the polymorphonuclear leucocytes especially seem very sticky.

On two occasions we have observed a small round homogeneous body, which we have taken to be of the same nature as one of the larger food bodies, become attached to the posterior end of an amoeba and instead of being cast off after a time, the amoeba has gradually taken the body into its endoplasm. We have never observed the intake of a polymorphonuclear leucocyte in this manner nor have we ever seen anything in our stained preparations suggestive of a whole ingested polymorphonuclear leucocyte within an amoeba.

There is no contractile vacuole.

We agree entirely with Craig when he says that in the vast majority of cases the nucleus is invisible in the living organism. On only two or three occasions have we satisfied ourselves that the round body

within the endoplasm which we have taken for the nucleus has been that structure. When an amoeba shows numerous round inclusions, as is often the case, it is impossible to say that any particular round body in the endoplasm is the nucleus.

(b) *The stained organism.*

When stained with iron-haematoxylin the ectoplasm of the amoeba stains grey and appears finely granular in structure in protruding pseudopodia. The endoplasm is often sharply marked off from the ectoplasm and appears to be somewhat coarsely granular in structure and frequently shows an alveolar appearance. Usually one finds inclusions of various shapes and sizes within the endoplasm and these are often present in sufficient numbers to obscure the structure of the endoplasm.

The nucleus has been fully and accurately described by Craig who deals also with the differences and similarities between it and the nuclei of *Entamoeba coli* and *Entamoeba histolytica*. It consists essentially of a circular or oval nuclear membrane separated by a clear zone from a small centrally situated karyosome. The membrane seems to be impregnated with a certain amount of chromatin for it stains deeply with iron-haematoxylin and also frequently bears granules or small thickenings of chromatin on its inner side. The karyosome may appear to consist of a single body homogeneous in structure and this is most commonly found, or it may very frequently appear to be made up of three or four distinct granules. In several of our preparations a karyosome appears to be absent and the space within the nuclear membrane is taken up with a net-work of linin fibres on which small granules of chromatin are irregularly disposed. It is possible that such appearances may represent stages in the break up of the chromatin prior to nuclear division, though none of the amoebae show anything which can be interpreted as mitotic division figures.

The nucleus is extremely poor in chromatin, and only stains feebly with chromatin stains such as safranin, methyl-green, polychrome methylene-blue. There is thus a very marked contrast in appearance between it and the round or oval inclusions, many of which are coloured intensely with the stains just mentioned. Pl. XX, fig. 2 is a photomicrograph of an organism stained with safranin and shows how feeble is the contrast between the cytoplasm and the nucleus.

Wenyon (1907) speaks of the paucity of chromatin in the nucleus of *Entamoeba muris*, and in this connection also we may note that a

section of an intestinal ulcer showing *Entamoeba histolytica* which was kindly lent to us by Dr J. H. Ashworth, showed the cytoplasm of the amoeba stained blue with methylene-blue whilst the nucleus was stained pink with eosin. One generally thinks of nuclei as basophilous and not eosinophilous.

Nuclear division must take place but so far we have not found any organisms which show any stage of the process.

We have found one bi-nucleate amoeba which is shown in Pl. XX, fig. 6, and on one film we were fortunate in obtaining a late stage in the fission of an amoeba, shown as a photomicrograph in Pl. XX, fig. 7.

So far, we have not been successful in finding either the cystic or the pre-cystic stage described and figured by Craig.

Our measurements of the amoeba and the nucleus agree on the whole with those given by previous workers. One of the smallest forms we have found is that shown in Fig. 5 and measures 7.5μ in its longer axis. The organisms vary from this up to about $25-27\mu$ when in an extended condition. We have not found any of the large forms mentioned by Smith and Barrett reaching 60μ in length. The nucleus measures from about 2.5μ to 4μ in diameter.

(c) *The Food of Entamoeba gingivalis.*

The importance of determining the nature of the food of *E. gingivalis* need not be emphasised here except to point out its relevancy to the question of the possible pathogenicity of the organism.

Reference to the literature dealing with this subject yields no definite information on the point. Doflein (1911, p. 595) says that the amoeba feeds chiefly on leucocytes and bacteria, whilst Hartmann (1913, p. 641) says that the food consists of bacteria of the mouth cavity, but chiefly of leucocytes and leucocyte remains.

These statements appear to be based largely on the remarks of von Prowazek and Leyden and Loewenthal on the subject. Prowazek (1904) says that *E. buccalis*, which is undoubtedly the same organism as *E. gingivalis*, lives among leucocyte masses, and takes in this food (presumably the leucocytes) by ectoplasmic engulfment. He goes on to describe the process of digestion, as revealed by the aid of neutral red, and the throwing out of the undigested nuclear remains.

Leyden and Loewenthal (1905, p. 9), in speaking of the amoebae found by them on the surface of a cancer inside the mouth say that the food appears to consist chiefly of leucocytes, with whose remains they are frequently completely filled, whilst bacteria are seldom found in

food vacuoles. They go on to state that the food bodies in the amoebae exhibit the staining reactions of nuclear substance with haematoxylin and iron-haematoxylin.

We may usefully point out here what has been mentioned already, that in none of our observations either on the living organisms or on stained material have we found any evidence whatever in support of Prowazek's statement that the amoeba ingests leucocytes, if by this word is meant polymorphonuclear leucocytes.

Smith and Barrett (1915 *a*, p. 166) say that in the endosarc there are many digestion vacuoles in which globular detritus of leucocytic nuclei and red blood cells are commonly found along with bacteria.

Craig (1916, p. 230) says that not all the ingesta are of leucocytic origin, and puts forward the suggestion that those round bodies which stain deeply with iron-haematoxylin and are surrounded by a clear zone, represent some form of yeast or protozoan organism, and are not of leucocytic origin. He also mentions that it has not been his experience to observe ingested red corpuscles in the endoplasm, even where the surrounding medium contains red blood cells.

Colyer (1916, p. 52) gives points for and against the amoeba being the cause of the disease. Among the points in its favour he speaks of "its power to phagocyte red cells, a power not possessed to any degree by free-living amoebae."

From this short account of previous work on the subject it will be seen that there is no agreement as to the real nature of the ingesta, except that most of the authors mention leucocytes or leucocytic remains, whilst two consider that red cells may be among the ingesta. The word leucocyte has a wide connotation, and it was therefore necessary for us to carry the matter still further, and obtain as far as possible accurate information on this point. We have made a careful attempt to determine the real nature of these bodies, because we realised that upon the elucidation of this point depended, to a large extent, the answer to the question whether or no *E. gingivalis* is a true parasite of pathogenic importance.

The ingesta within the amoeba are seen to consist principally of two constituents, bacteria and larger bodies of various shapes and sizes, but most frequently round or oval in outline. These bodies are by far the commonest constituent of the ingesta, though one may occasionally encounter amoebae without any food bodies at all. Again, other amoebae are found showing only bacteria within them (Pl. XX, fig. 3), either lying singly or in groups within vacuoles, and one not uncommonly

finds organisms in which both bacteria and the larger bodies occur together, as shown in Pl. XX, fig. 4. The round bodies present a greenish appearance in the live amoebae, and when stained appear to be almost homogeneous in structure. They frequently show a narrow clear zone around them, separating them from the inner limit of the vacuole, but as often seem to occupy the whole area of the vacuole.

With iron-haematoxylin, the majority of them stain an intense black whilst others exhibit various degrees of blackness, and some are only faintly stained.

The black coloration with iron-haematoxylin suggests that they are composed of a chromatinic substance, a fact which is confirmed by the use of other stains. Safranin stains the bodies bright red, Unna's polychrome methylene-blue stains them deep blue, methyl-green colours them green, whilst with Giemsa's stain they are tinted a bluish purple or purple, and Jenner's methylene-blue eosin stains them blue or greenish blue.

All these stains, therefore, show that the bodies are rich in chromatin, and that they are probably derived from nuclear material. They also definitely rule red blood cells out of the question.

This is important, for it shows that the bodies are not of the same nature as the ingesta of *E. tetragena*, in which red blood corpuscles are frequently found.

Nor are whole polymorphonuclear leucocytes ever to be found within the amoebae, although endothelial cells showing ingested polymorphonuclear leucocytes are frequently observed in material from pyorrhoea pockets or from inflamed areas of the gums (Pl. XX, fig. 8).

In size the bodies vary from about 1μ to 4 or 5μ in diameter, our measurements agreeing with those given by Craig.

Having satisfied ourselves of the chromatinic nature of the deeply staining bodies we set out to discover their origin. It was evident that they were of common occurrence in the mouth, and were not confined to pyorrhoea pockets although they were to be found in amoebae from these situations as well as from any other part of the gums or necks of teeth. We were not satisfied that they were derived from polymorphonuclear leucocytes, for none of these ever showed nuclei of the homogeneous type and having the same staining reactions as the round bodies. We were, therefore, driven to make as thorough a search as possible for the source of the round bodies.

Careful examination of our films carrying amoebae showed that, scattered about amongst the innumerable bacteria and leucocytes,

there were here and there small bodies having exactly the same shape, size, and general appearance as the ingesta of the amoebae. It was clear that if we could satisfactorily explain the nature and origin of these bodies we should be in a position to clear up the question of the ingesta of the amoeba.

The clue to the solution of this problem was obtained when it was observed that on one of our films certain of the bodies within the amoebae, and scattered about in the film, possessed small fragments of cytoplasm attached to the deeply staining nuclear substance of which they were composed (Pl. XXI, fig. 13). It was further noticed that occasionally the bodies occurred in groups of two or three (figs. 13 *a*, *b* and *c* and 14 *a-m*), and this, together with the occurrence of the cytoplasmic remains attached to the bodies suggested that they were produced by the disintegration of some form of polymorphonuclear leucocyte.

It was known that in the saliva there occurred the salivary corpuscles, which could be observed undergoing disintegration and dissolution, when saliva was examined on the warm stage.

Film preparations were therefore made from saliva and from the tonsils, from which the salivary corpuscles are poured out, and were fixed and stained in exactly the same way as the films for amoebae.

Examination of these films, salivary and tonsillar, revealed salivary corpuscles in an almost endless variety of degeneration and disintegration, and it was found that round bodies exactly similar to the ingesta of the amoebae and to those scattered about on the amoebae films were present on these films also.

It was at once apparent that the bodies in question were produced from the nucleus or nuclei of degenerated and disrupted salivary corpuscles.

The latter were found to present a variety of nuclear appearances; some were mononuclear, others showed all kinds of transition stages between the purely mononuclear and the polymorphonuclear condition. The size also varied greatly. Those showing the polymorphonuclear condition had a very different appearance especially in the lobes of the nuclei from the true phagocytic polymorphonuclear leucocyte which is also very common in the mouth. The photomicrographs, shown in Figs. 9 and 9 *a*, illustrate the differences very well¹.

¹ N.B. It is not within the province of this paper to go into the question of the evolution of salivary corpuscles within the body nor into their relation to the leucocytes of the blood. Reference should be made to the writings of Weidenreich (1908 and 1911) for discussion of these subjects.

Our films show that when the salivary corpuscles undergo dissolution in the saliva and in any situation in the mouth, their cytoplasm becomes coarsely granular in appearance, and is finally ruptured and thrown off from the nuclei which may remain with fragments of cytoplasm attached to them, or may be left completely naked. The nuclei also stain in a great variety of ways. Some of them appear to swell up and only stain faintly, others seem to remain normal in size, and some apparently shrink a little and are stained an intense black with iron-haematoxylin. The majority of them appear to become practically homogeneous in structure.

Not all the nuclear lobes of a given corpuscle behave in the same way, one portion may be pale and the other or others quite black. The series of drawings shown in Pl. XXI, fig. 14 *a-m* gives a small selection of disintegrating salivary corpuscles and their products taken from films of saliva and tonsillar smears.

A comparison of these with the accompanying drawings of amoebae with their ingesta will at once show the identity of the bodies in question. It would be easy to multiply the drawings indefinitely and so accumulate still more evidence in support of our suggestion, but we think that the figures given are sufficient to illustrate our point.

Salivary and tonsillar smears stained by other methods than iron-haematoxylin, as for example with Giemsa's or Jenner's stains, also point conclusively in favour of the explanation we have advanced.

It is easy to see how, with the extrusion of salivary corpuscles and their subsequent disintegration always going on, a supply of their nuclear remains can accumulate in the mouth, on the gums or at the bases of the teeth, in fact at any point where a suitable lodgment can be found, and how the amoebae are continually furnished with fresh supplies of food by this means.

Our explanation of the nature of these bodies is at once simple and feasible. It is, further, easy to understand the extreme prevalence of the bodies among the ingesta of the amoebae, and at the same time shows that the organism is of no pathogenic significance, but is on the other hand a scavenger of naturally occurring waste nuclear material together with bacteria occurring in the mouth. It may therefore be considered as a useful rather than a harmful organism.

As has been mentioned earlier, previous investigators have suggested the leucocytic nature and origin of the ingesta under discussion but the point which we make and one that has not been suggested before is

that the bodies are the product of certain definite leucocytes, namely the salivary corpuscles.

Craig's suggestion mentioned above, that the black bodies surrounded by a clear zone are some protozoan or yeast-like organism, receives no support from our observations, and seems to us unnecessary. We do not wish to assert that yeasts may never be ingested by the amoebae, for we have found a few small yeast-cells on smears made from the healthy gums of the child of one of us (T. G.). A few amoebae are present on these films, and show the usual round deeply staining bodies amongst the ingesta but not yeasts. The bodies under discussion are also quite distinct in appearance from the yeast-cells which may frequently be found ingested by *Entamoeba coli*, in smears made from faeces. A drawing of one of these is shown in Pl. XXI, fig. 15.

DESCRIPTION OF CASES.

1. Child, male, aged 2. Healthy mouth and gums. Smears taken from gum margin. Amoebae present.

2. Girl, aged 5. Sent by Dr Waller from the Birmingham General Hospital to the Dental Hospital, with the extremely rare condition in the deciduous dentition of the progressive loosening and loss of temporary teeth, one after another in both jaws, the teeth being shed with no absorption of the roots whatsoever. The disease appeared to commence in the gum, extending to the bone of the socket and the periodontal membrane, being finally cast off with the total destruction of the latter structure. The condition is analogous to pyorrhoea alveolaris in the permanent dentition.

The amoeba was found in the discharge squeezed from between the gum and an affected tooth; and amongst the *débris* collected from the end of the root of the lower right canine which was loose enough to be picked from its socket with the fingers.

3. Boy, aged 13. Teeth in a shocking state of irregularity, and very carious. He had been wearing appliances for the regulation of teeth for some time. The mouth was very badly kept, *materia alba* accumulating over the buccal and labial surfaces of the teeth producing very rapid and extensive caries. The gums were inflamed on the buccal and labial surfaces of both jaws but no pockets existed. Amoebae not present.

4. Girl, aged 10. Mouth well-kept, gums healthy. An orange-stained deposit on the labial surface of the erupting lower right canine was examined, but no amoebae were found.

5. Boy, aged 13, with inflammatory hypertrophy of the gum on the labial aspect of the maxillary incisors. Material was collected from beneath the enlarged gum festoons and was found to be very rich in amoebae. The hypertrophied tissue was removed, fixed in Bouin's solution and embedded in paraffin. Serial sections were cut, stained by a variety of appropriate methods, and carefully examined for the presence of amoebae with a negative result.

6. Girl, aged 16. Teeth in fairly good condition but the interdental gum papillae were somewhat enlarged and inflamed, due to the retention of food *débris*. Films made from the *débris* in the "trough" between the papilla and the tooth showed the presence of amoebae.

7. Female, aged 21. Teeth well-kept and mucous membrane of the mouth generally healthy, but the margin of the gum around the upper right maxillary first molar was irritated by a small collection of salivary calculus, and had reached a stage of chronic inflammation. Films made from the material beneath the inflamed gum, and others made from healthy gum showed no amoebae.

8. Male, aged 31. Mouth very well kept. Singularly free from dental caries. Case of true pyorrhoea alveolaris affecting chiefly the upper and lower incisor regions, the left lower molars, and to a slight extent the left maxillary canine (lingual aspect only) and premolars. In the infected areas the gum had a tense, glistening appearance and was of a deep bluish-red colour, deepened over the region of the pockets, so that one could distinguish by the colour of the gum the positions where the greatest amount of bone destruction had occurred. The interdental papillae were retracted; not removed by ulceration as one sees in some conditions, but drawn down tightly over the interdental bony septum, becoming more and more retracted, as more and more bone was destroyed. From the pockets, a fairly copious amount of pus could be expressed at almost any time; and on the roots of the teeth corresponding to the position of the retracted gum a deposit of hard black calculus was found, which in some places extended along the root for a short distance into the pocket. Films were made from the pus from around the upper incisors, but we did not detect the presence of the amoeba until we had examined upon the warm stage some of the material from the mandibular incisors. At no time were the amoebae very numerous, but the most favourable moment for them was in the first expressed bead of pus which would be contaminated with a certain amount of *débris*.

We cannot agree with the statement that the amoebae are most numerous at the bottom of a pyorrhoea pocket. Our experience proves

the contrary, namely, that if precautions are taken to avoid contamination with the superficial *débris*, material collected from deep pockets is less rich in amoebae than that collected from a more superficial area.

9. Male, aged 34, with mouth in a very unhygienic condition. The buccal and labial surfaces of the teeth were badly discoloured, some of the posterior teeth being coated with salivary tartar, whilst all the remaining molar and premolar teeth had a rim of hard calculus at the cervical margin on both the buccal and lingual surfaces. There was a marginal gingivitis in both jaws, but no suppuration could be detected except around the left mandibular second molar, where it was very slight. The interdental papillae were reduced in size and inflamed. There were no distinct pockets, but in places the alveolar border and gum had shrunk, leaving the neck or the root of the tooth exposed.

The amoeba was found on both the salivary calculus and the rim of hard dark calculus at the gum margins; amongst the white deposit on the buccal surfaces of the molars, and in the *débris* beneath the inflamed gum margins.

10. Male, aged 37, emaciated, thin and anaemic, suffering from chronic rheumatism. He was sent from a General Hospital for dental treatment. His mouth was in a very neglected state, with several badly broken-down septic roots, the buccal surfaces of the remaining posterior teeth covered with salivary calculus, and the usual rim of hard dark tartar associated with marginal gingivitis at the necks of the anterior teeth, and on the lingual aspect of the necks of the posterior teeth. The marginal gingivitis was very pronounced. Hanging-drop preparations and stained films from both kinds of calculus, and from the matter expressed from the gum margins showed abundant active amoebae.

11. Female, aged 40. Recession of gum around buccal roots of left maxillary first molar. No pus present, and no pockets. Films made from *débris* on the exposed roots showed amoebae.

12. Female, aged 38. Recession of gum from buccal and distal surfaces of left upper maxillary second molar due to presence of calculus, but there was no visible suppuration. Stained films showed the presence of amoebae.

13. Male, aged 43. Pyorrhoea affecting all remaining maxillary teeth including the incisors, canines and second molars. There was recession of the gums all round the teeth and the formation of pockets on the lingual aspects of the incisors and canines and the mesial surfaces of the molars. The whole of the palatal surface of the gums, periosteum, and that between the canines and second molars was in a

"boggy" condition to the touch. Suppuration was not constant and had been kept fairly well in check by periodical local treatment. Occasionally a pocket would get infected, giving rise to acute inflammation and the formation of a local abscess. Amoebae present.

14. Male, 49. Similar to "13." Films also showed presence of the organism.

15. Male, aged 43. Films made from the material collected in the "trough" existing between the normal healthy interdental gum papilla and the tooth showed the presence of amoebae, flagellates and numerous active phagocytic leucocytes.

16. Male, 36. Recession of the gum on labial surface of root of the first left lower incisor exposing more than one-third of the root. Material collected from the exposed root contained amoebae, as did also material from a rather deep "trough" existing between the distal surface of the mandibular second left molar and the gum.

17. Female, aged 30. Inflammation of gums chiefly affecting the labial and buccal aspects and the interdental papillae of both jaws. There was some recession of the gums about buccal surface of right premolars and first molar, but no deep pockets were to be found and there was a tendency to hypertrophy of the interdental papillae rather than resorption. The whole of the affected gum surface was of a deep red colour, bleeding freely to the slightest touch and very painful. Material collected from between the inflamed gum and the tooth surface contained numerous amoebae.

18. Male, 52. Leucoplakia of the cheeks, affecting both sides. On right side it seemed to extend from inflammatory condition of gum around second mandibular molar. Films made from about the molar, and the surface of the cheek did not show any amoebae.

19. Male, 55. Chronic condition of the tongue in which the sides of the anterior third and the tip were liable to recurrent attacks producing painful, raw patches. No amoebae.

20. Male, 50. All teeth were in a perfect condition except right maxillary first molar which was missing. The mouth was exceedingly well kept and the gums generally speaking were in a good condition, with the exception of slight recession of the buccal margins about the premolar and molar teeth in both jaws and some evidences of an inflammation of the interdental papillae here and there.

Films made from the "troughs" existing between the interdental papillae and the teeth showed the presence of amoebae in the case of both healthy and unhealthy papillae.

21. Male, 38. So-called dental ulcer on the lingual aspect of the left mandibular gum, caused by the chafing of a denture. Film made from its surface gave negative results so far as amoebae are concerned.

22. Male, 30. A case of marginal gingivitis commencing in the interdental papillae, producing rapid sloughing of the latter and extending along the gum margins. Amoebae very numerous.

23. Male, aged 28. Right mandibular second molar which had been capped with gold became very painful and loose, with a good deal of inflammation in the gum around it. After extraction the *débris* collected on the surface of the gold and on the roots was examined but no amoebae were found.

24. Male, aged 44. A "bridge" attached by gold caps to the left maxillary first premolar and the third molar on the same side, was extracted together with the abutment teeth, on account of the loosening of the latter by the gradual destruction of the bone and resorption of gum. No discharge was present. *Débris* from the roots, from the surface of the gold caps and from under the surface of the gold "bridge" was examined and in no case were amoebae found.

Summarising the results, we have found *Entamoeba gingivalis* present in the majority of cases of pyorrhoea, but not in all. It is also present in most cases of gingivitis not associated with alveolar absorption, although here also in some of our cases the organism is absent.

In some mouths with local evidences of recession of the gum associated with alveolar absorption, the amoeba was found in the diseased positions, and also in the healthy positions in the same mouths. We have found it in a case of marked hypertrophy of the gum as well as in cases of rapid sloughing of the margins of the gums and the interdental papillae.

The organism was present in the healthy mouth of a child of two years old, and also in that of a man of 43. It was not present in an unhealthy mouth of a boy, aged 13, with very irregular teeth, most of which were rapidly succumbing to dental caries; and whose gums were also inflamed and bled easily (No. 3). Neither was it present in an orange-stained deposit on the labial surface of a lower canine in a child of 10 (No. 4). In three other pathological conditions in the mouth (Nos. 18, 19, 21) we have not found the organism, although cases of aphthous and ulcerative stomatitis and glossitis examined by Lynch (1915) gave positive results.

We conclude from our examinations of the above cases that *Entamoeba gingivalis* is not the cause of pyorrhoea alveolaris, since,

although common in the deposits around the teeth in this condition, it is by no means always present. Further, its presence in other pathological conditions of the oral muco-periosteum, and also under certain circumstances in healthy mouths does not support the claims of Smith and Barrett and others; but on the contrary seems to suggest that it is dependent upon a factor common to all these cases. That factor is, in our opinion, the existence of a "trough," or an interdental space, or a pocket, or a rough surface such as that afforded by an accumulation of tartar, or other place inaccessible to the natural cleansing agencies of the mouth, where food particles and cellular *débris* and bacteria can collect, and where the amoeba exercising its (in our view) beneficent function of scavenger of dead nuclear matter and bacteria, would find plenty of sustenance.

The fact that it is not found in all such cases (see Nos. 3, 4, 7, 23 and 24) we are not at present able to explain; possible explanations are: (1) The influence exerted upon the amoeba by the reaction of the saliva. (2) The kind of food accumulating, whether proteid or carbohydrate were in excess, would determine the kind of change going on in the accumulation, *i.e.* putrefactive (alkaline) or acid-production. (3) The variations in the mouth flora¹. Cases 3 and 4 in which the *débris* collected from the tooth surfaces was strongly acid seem to suggest that the mouth amoeba has no great fondness for an acid medium.

CONCLUSION.

Bringing together the evidence derived from the two sources, viz. (1) the cytological investigation into the nature of the ingesta of the amoeba, and (2) the examination of a variety of oral conditions, as to the relation of *Entamoeba gingivalis* to Pyorrhoea Alveolaris, we find that the first leads us to the conclusion that the organism so far from being a destroyer of healthy tissues in the mouth is in reality a devourer of waste nuclear material derived from disintegrated salivary corpuscles together with bacteria and is therefore most probably a useful scavenger, whilst the second leads us to the conclusion that the amoeba may be found in healthy and unhealthy mouths and is especially likely to be found in situations where there is the possibility for the accumulation of food *débris*.

Our general conclusion is that there is no evidence to show that *E. gingivalis* is the cause of disease.

¹ These of course must depend largely upon the nature of the oral secretions and the character of the food.

A NOTE ON THE TRICHOMONAD FLAGELLATE

TETRATRICHOMONAS BUCCALIS N. SP.

BY T. GOODEY.

In several of the recent papers dealing with *Entamoeba gingivalis*, mention is made of the occurrence in preparations from the mouth of a trichomonad flagellate. One writer, Lynch (1915), names it *Trichomonas vaginalis* evidently identifying it with the flagellate bearing that name. Other writers adopt the course followed by the text-books of including the organism under the name *T. hominis* or *T. intestinalis* and so identifying it with the human intestinal form. The only published figures showing the appearance of the organism are those attributed to v. Prowazek which are however very unsatisfactory. In the light of these considerations the following note is put forward as an attempt to present some accurate information as to the appearance and structure of the organism.

The flagellate was found fairly plentifully in material consisting of food *débris*, etc., removed from between the lower incisors of one of the cases examined by us. Hanging-drop preparations were made and examined on the warm-stage, and though at first the flagellates did not appear to be numerous, after a short time they were found in fairly large numbers, actively moving about in the drop.

Their movements were very rapid, and it was impossible to determine anything concerning the number and movement of the flagella, neither could it be ascertained whether an undulating membrane was present.

The posterior end was frequently seen to be much drawn out into a long tapering point, and the organisms often displayed great amoeboid activity, sending out pseudopodial extensions, both anteriorly and posteriorly.

The hanging-drops were fixed and stained so that the organisms could be studied in detail.

It was found that in staining with iron-haematoxylin the flagellates differentiated very quickly, so that by the time the leucocytes and amoebae on the films were differentiated, the flagellates were practically decolorised entirely. It was therefore necessary to differentiate for a short time, and by this means, satisfactorily stained flagellates were obtained.

When properly fixed and stained it is found that the body of the flagellate is longer than it is broad, varying in length from about 7μ to 12μ , and that the protoplasm of which it is made up is alveolate in structure.

Small bacteria are frequently to be seen lying in the protoplasm of the body. A mouth or cytostome could not be distinguished in any of the numerous organisms examined, but one may be present nevertheless. It is highly probable that the flagellate feeds by the ingestion of bacteria by means of pseudopodial activity, when undergoing amoeboid movement. Pl. XXII, fig. 21, showing a flagellate with numerous ingested bacteria in a much extended amoeboid condition, supports this suggestion.

The nucleus, which is oval or ellipsoidal in shape, lies towards the anterior end of the body. It seems to stain rather diffusely, and shows, so far as our preparations reveal its structure, no karyosome. Occasionally, a few rather more deeply staining areas can be distinguished in it.

In front of the nucleus there is a distinct round or oval blepharoplast, which is connected with the nucleus by means of a short rhizoplast.

The flagella are four in number, not three as in *Trichomonas intestinalis*, and arise from the blepharoplast. They are rather long and delicate, all appearing to be of about the same length, and stain faintly.

There is an undulating membrane which takes origin in the blepharoplast, and extends along one side of the body, to about two-thirds or three-quarters the length of the latter. There does not appear to be a free posterior flagellum to the undulating membrane, and in this respect it differs from *Trichomonas intestinalis*, and many other species of the same genus.

A chromatinic basal rod ("parabasal body" of Kofoed) appears to be present in connection with the undulating membrane, though it varies greatly in its extent and staining reactions. In one or two cases it appeared to follow the edge of the organism, whilst in others it seemed to be curved on to the surface, as shown in Pl. XXII, figs. 17 and 18. In one case it was represented by a row of granules, whilst in several it was not possible to distinguish anything at all corresponding to it, as shown in Fig. 19.

An axostyle is present, and extends from the region of the nucleus, where it seems to be somewhat expanded, in a posterior direction and frequently terminates in a sharp spike outside the end of the body, as in Pl. XXII, figs. 17-20.

In the amoeboid form shown in Fig. 21 it is seen as a straight rod. It stains rather more deeply than the cytoplasm of the body, and does not stand out as clear, almost unstained organ, as represented in the drawings of many species of *Trichomonas*, but resembles more the appearance of the axostyle figured by Mackinnon (1913) in *Tetratrichomastix parisii*.

The question of the naming of this organism presents certain difficulties.

It is, without doubt, the same trichomonad as that figured by Doflein (1911, p. 493) and by Jollos (1913, p. 693), the figures being taken from Prowazek. The finely tapering tail portion and the appearance of the undulating membrane prove this. Doflein calls it *Trichomonas hominis*, and Jollos *T. intestinalis*; each identifying it with the trichomonad from the intestines, and explaining its different appearance from that form as being due to the influence of different environmental conditions. Whether this is a sufficient explanation of the morphological differences presented by the mouth and intestinal forms is, in the writer's opinion, rather doubtful. The great variety of shape exhibited by the mouth form, as compared with the fairly constant oval or pyriform shape of the intestinal form, is one point of difference.

Again, in the mouth form, the undulating membrane is no stouter than the anterior flagella, and does not terminate in a free posterior flagellum, nor is the basal chromatinic rod connected with it well developed, whereas, in the intestinal form the undulating membrane is stouter than the anterior flagella, and there is a posterior flagellum and a well-developed basal rod.

The nucleus also and the axostyle, in the two forms, present further points of difference. In the mouth form, the nucleus seems to stain rather diffusely, and distinct chromatin granules are very rarely discernible, whereas, in the figures of the intestinal form chromatin granules are shown. The axostyle in the mouth form stains rather darker than the cytoplasm whilst in the intestinal form it appears as a clear refractile bar, according to Wenyon (1915).

Unfortunately, it has not been found possible to make a careful comparison of the mouth form described above with good examples of the intestinal form. The latter was obtained in faeces smears on one occasion, but the majority of the organisms present were in a degenerate condition; some of them showing in life the production of small lateral pseudopodia, characteristic of the dying condition. The few that were

found to present a normal appearance on the stained films agreed well with the illustrations of the intestinal form, as figured by Brumpt (1913).

Judging then by the aid of the published figures of the intestinal form it is in the writer's opinion preferable to separate this from the mouth form, and not unite them under the specific names of *hominis*, as done by Doflein, or *intestinalis* by Jollos.

The names *hominis* or *intestinalis* should be employed for the intestinal form alone (the former has the priority claim) whilst the writer proposes the specific name *buccalis* for the mouth form, at any rate provisionally until such time as further research can prove that the important morphological differences presented by the two forms are merely due to different environmental conditions.

The fact that the mouth form described in this note, possesses four anterior flagella, does not affect the question of specific difference; it merely shows that it should be placed in the subgenus *Tetratrichomonas* created by Parisi (1910). *Tetratrichomonad* and even *pentatrichomonad* forms occur in the intestines according to Wenyon (1915), though very rarely.

The name therefore proposed for the flagellate in question is *Tetratrichomonas buccalis*, n. sp.

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EXPLANATION OF PLATES XX—XXII

PLATE XX.

Photomicrographs, all magnified 1325 diameters, except Fig. 7, which is magnified 820 diameters.

Fig. 1. *Entamoeba gingivalis*, trophic form showing characteristic ingesta; the nucleus is seen on the left side of the amoeba as a ring of chromatin with a central granule. Stained. Iron-haematoxylin.

Fig. 2. *E. gingivalis* form stained with safranin and licht-grün, and showing the feebly stained nucleus as a ring with central karyosome. The nucleus on the right side just overlies a more deeply stained food body which is slightly out of focus.

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13a

13b

13c

14a

14b

14e

14d

14c

14f

14g

14h

14i

15

14j

14l

14m

14k

16

- Fig. 3. *E. gingivalis* forms showing bacteria ingested as the source of food. Stained. Iron-haematoxylin.
- Fig. 4. *E. gingivalis*, trophic form showing characteristic round bodies within food vacuoles, and rods of bacteria at posterior end. Iron-haematoxylin.
- Fig. 5. *E. gingivalis*, very small form 7.5 μ long, from pyorrhoea material from child of 5 years. Iron-haematoxylin.
- Fig. 6. *E. gingivalis*, bi-nucleate form with the nuclei in focus, the cytoplasm is out of focus. Iron-haematoxylin.
- Fig. 7. *E. gingivalis*, late stage of fission, the connecting strand of protoplasm is slightly out of focus. Iron-haematoxylin.
- Fig. 8. Endothelial cell with an ingested polymorphonuclear leucocyte towards lower end slightly overlapping the cell nucleus. Iron-haematoxylin. From pyorrhoea pus.
- Fig. 9. Polymorphonuclear leucocyte from gum, ingesting a rod of bacteria and showing the open character of the lobed nucleus. Fixed absolute alcohol, stained methyl-green.
- Fig. 9a. Polymorphonuclear salivary corpuscle showing the rounded almost homogeneous lobes of the nucleus. Stained iron-haematoxylin.

PLATE XXI.

Camera lucida drawings, all magnified 2725 diameters. All stained with iron-haematoxylin.

- Figs. 10, 11, 12, 13, drawings of *E. gingivalis* showing characteristic inclusions of various shapes and sizes and staining reaction. Fig. 13 shows the cytoplasm of the amoeba in an alveolate condition, whilst the inclusions show small pieces of cytoplasm attached to the chromatinic material. Fig. 13a, b and c, deeply stained nuclear lobes with attached cytoplasmic fragments from same film as Fig. 13.
- Fig. 14a-m, disintegrating salivary corpuscles and their products, showing the same characteristic appearances as the amoeba inclusions, drawn from salivary and tonsillar smears and from pyorrhoea pus and food debris smears.
- Fig. 15. *Entamoeba coli*, a small trophic form showing two ingested yeasts lying in one vacuole, note the different appearance of these from the inclusions in *E. gingivalis*.
- Fig. 16. Outline of red blood corpuscle drawn to the same scale of magnification for purposes of comparison.

PLATE XXII.

Tetratrichomonas buccalis n. sp. Camera lucida drawings magnified 4100 diameters. All stained with iron-haematoxylin.

- Figs. 17-20. Four different free swimming trophic forms showing the four anterior flagella, the undulating membrane and the relations of nucleus, blepharoplast and rhizoplast, and also the axostyle. In Fig. 19, the chromatinic basal rod connected with the undulating membrane appears to be absent.
- Fig. 21. An organism in a much extended amoeboid condition showing numerous ingested bacteria and the axostyle as a long narrow rod. The outline of the nucleus is lost, owing to the differentiation having been carried rather too far.