THE HUMAN INTESTINAL AMOEBA, IODAMOEBA WILLIAMSII, AND ITS CYSTS (IODINE CYSTS).*

By W. H. TALIAFERRO, PH.D., AND ELERY R. BECKER, A.B.

(Received for publication December 29, 1921.)

PLATES VIII AND IX.

CONTENTS.

Introduction	188
Historical review.....	189
Material and methods.....	193
The motile amoeba.....	193
The cyst.....	198
Nomenclature	203
Conclusions	205
Bibliography	206

INTRODUCTION.

The "iodine cysts" which Wenyon originally described as occurring in human faeces have now been found by a number of investigators. Not only have they been found in human faeces, but O'Connor (1920) and Chauchemez (1921) have noted the presence of an "iodine cyst" in the intestinal contents of the pig which is indistinguishable from the human form. These peculiar bodies differ markedly from the common intestinal cysts of man. While a large number of them are spherical in shape, some present very bizarre and irregular outlines, and in freshly passed cysts the cytoplasm contains a large inclusion of glycogen which takes the typical mahogany-brown stain when observed in iodine. The mature cysts are typically uninucleate, and, as we will see later, the structure of this nucleus is different from that of the other intestinal cysts.

While there is no doubt at the present time that the "iodine cysts" represent the encysted stage of an amoeba, there is considerable variance of opinion as to, first, the systematic relationship of this amoeba, and second, the structure of the nucleus, both in the motile amoeba and in the cysts. The object of the present investigation was

* From the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.

to make a careful study of both the motile and encysted forms, with especial reference to the structure of the nucleus. With this background we hope to be able to correlate better the findings of different investigators, and to discuss the nomenclature of the species. The most interesting thing in the present work is that, although our findings support the contentions of Dobell (1919), we have been able to duplicate the findings of other investigators by using different microscopical methods.

HISTORICAL REVIEW.

As stated above, these cysts were first observed in human faeces by Wenyon (1915) who described them as "spherical bodies" with an "iodophilic" inclusion. A year later the same author re-described them under the name of "iodine cysts" or "I. cysts." In his first paper he suggested that they were probably vegetable in nature, and in the second paper he believed that he had seen them germinate when kept in faeces. In a still later paper Wenyon and O'Connor (1917) state that besides the typical "iodine cysts" there are sometimes present larger spherical bodies which appear homogenous, with a central vacuole, which, they believed, probably "represent large I-cysts which are devoid of iodophilic bodies or structures of an allied nature."

Kuenen and Swellengrebel (1917) found the "iodine cysts" and an active amoeba, which very probably was the active amoeboid form of the cysts. They provisionally named this active amoeba, "Pseudolimax."

Matthews (1918) again described and figured "iodine cysts." This investigator followed Wenyon's suggestion that they were probably of a vegetable nature.

Flu (1918), in the same year, came to the conclusion that these cysts were, in reality, degenerate cysts of *E. tetragena* (*Endamoeba histolytica*).

After reconsidering the whole matter, Brug (1919) came to the conclusion that the "iodine cysts" are the encysted stage of an amoeba described by Prowazek (1911) under the name, *Entamoeba williamsi*. He was also of the opinion that this amoeba should be placed, with *E. nana*, in the genus *Endolimax* Kuenen and Swellengrebel, 1917. In this paper Brug gives a description of the nucleus of the motile amoeba as well as the cysts. The noteworthy feature of

this description is that he described, in the nucleus of the cysts, besides a large eccentric karyosome, a crescent-shaped mass of chromatin granules. We will take up a discussion of this description later.

In the same year Kofoid, Kornhauser and Swezy (1919) came to the conclusion that Wenyon and O'Connor's "iodine cysts" include two different organisms. They came to the conclusion that the homogeneous bodies seen by these investigators and considered by them to be "iodine cysts" devoid of their iodophilic inclusions are "the non-nucleated, homogeneous spore-like cysts of some vegetable organism, possibly the chlamydo-spore of some phycomycete of unknown affinities," and that the large glycogen-laden "iodine cysts" are uninucleate cysts of *Endolimax nana*. It is not necessary to consider these authors' conclusions in regard to the homogeneous bodies included by Wenyon and O'Connor in the "iodine cysts," because we feel that these bodies are probably not typical "iodine cysts." It is with the second conclusion that we are most interested, namely, that the "iodine cysts" are the uninucleate cysts of *Endolimax nana*. As we understand it, these authors base this conclusion on the following points: In the first place, it is known that the common intestinal amoebae (*E. histolytica*, *E. coli*, and *E. nana*) often have large glycogen masses during the early one- and two-nucleate stages of their cysts, and if the "iodine cysts" are, in reality, uninucleate cysts of *E. nana*, it is not surprising to find a large glycogen mass in them. In the second place, they maintain that the nucleus of the "iodine cysts" is similar to that of an uninucleate *E. nana* cyst in respect to: (a) dimension of nucleus, (b) position of nucleus in cyst, (c) structure, both as regards nuclear membrane, and arrangement and distribution of the chromatin. And in the third place, they maintain that there is an intergradation in size of the glycogen mass found in the "iodine cysts" and in uninucleate cysts of *E. nana*. Besides the question of the structure of the nucleus, which will be discussed in detail in a later section, there are two difficulties in the way of accepting the conclusion of these authors. The first of these is that the "iodine cysts" are, on the average, larger than those of *E. nana*; and the second is that these authors were unable to find any of the larger cysts with four nuclei which should occur, if these were cysts of *E. nana*. As a matter of fact, the great majority of the "iodine cysts" are uninucleate, and it is only rarely that a specimen with even two nuclei is seen. That these difficulties were clearly

realized by these investigators is shown by the following quotation: "In fact two nuclei are found in the larger cysts and, as yet, we have not found four in them. It seems probable that there may be a decrease in the size of the cyst, as the glycogen decreases in volume and as the nuclei increase from two to four." It is very difficult to accept this latter suggestion, because we have no evidence that once an amoeba secretes a cyst wall about itself that this cyst wall ever diminishes in size. Certainly in the case of *E. coli* we do not find any decrease in the size of the cyst as the organism uses up its mass of glycogen. This is true, although the binucleate cyst of *E. coli* often contains an enormous mass of glycogen.

Dobell (1919), working independently and without the knowledge of Kofoed, Kornhauser and Swezy's work, came to a conclusion which is radically different from these investigators. He maintained that the "iodine cysts" are the cysts of an amoeba which was first described by Prowazek under the name of *Entamoeba bütschlii*. This is also the same amoeba which Kuenen and Swellengrebel (1917) provisionally named "Pseudolimax." As this name was not proposed according to the rules of nomenclature, and as it was clearly not an *Entamoeba*, Dobell proposed the name, *Iodamoeba*, for the genus. According to Dobell the nucleus of the motile amoebae possesses a large central karyosome which is surrounded by a layer of granules—the so-called "peripheral chromatin." These granules take both the nuclear and plasma stains, although they give up such stains as haematoxylin much quicker than the karyosome. In such haematoxylin stains they can be overstained, in which case they are often confused with the karyosome, or they can be completely decolorized, in which case they become invisible. Outside of this layer there is a well-defined nuclear membrane which may have granules imbedded in it. According to Dobell, when the amoeba encysts, the nucleus undergoes a remarkable change, in that the karyosome migrates to the edge of the nuclear cavity and the remaining space becomes filled with large granules, such as were originally arranged in a single layer around the karyosome.

Although Dobell's work seems to be a very thorough and painstaking study, his conclusions are not accepted by some of the recent authors. Thus, in a description of a new amoeba from the human intestine (*Councilmania lafleuri*), Kofoed and Swezy (1921) reiterate their former view that the "iodine cysts" are cysts of *Endolimax nana*. They state that they are unable, with their methods of

fixation and available stains, to demonstrate in the nucleus of *C. lafleuri*, the eosinophilic granules which Dobell describes for the amoeba of the "iodine cysts." They even suggest that, with this exception, there is enough in common with their description of *C. lafleuri* and Dobell's description of *Iodamoeba* to make it desirable to reinvestigate Dobell's *Iodamoeba* to see whether or not it should not be considered *C. lafleuri*. It is, however, a little difficult to see their grounds for this suggestion, as the range of size of the two species is not at all similar—*Councilmania* being a very large amoeba and *Iodamoeba* being a very small form.

Brug (1921) has also returned to the study of the amoeba of the "iodine cysts." His description of the amoeba and its cysts is essentially the same as that given in his earlier paper. He also still feels that the amoeba should be called *Endolimax williamsi* Prowazek, 1911. In regard to the nomenclature he has one very interesting new fact to offer, in that he has been able to examine Prowazek's original slides of "Entamoeba williamsi." He finds that these slides contain both *E. coli* and the amoeba of the "iodine cysts." He feels, therefore, that although *E. bütschlii* Prowazek, 1912, is entirely the amoeba of the "iodine cysts," *E. williamsi* Prowazek, 1911, is in part the same species, and that the latter name should have precedence over the former.

Nöller (1921) has been able not only to examine Prowazek's original slides of *E. williamsi*, but also of *E. bütschlii*. This examination supports the contentions of Brug. Nöller feels, however, that since Prowazek's description of *bütschlii* is based solely on the amoeba of the "iodine cysts," whereas the description of *williamsi* is based only in part on this amoeba, the sensible thing is to accept the former name. He therefore uses the name, *Iodamoeba bütschlii* Prowazek, 1912, emend. Dobell, 1919.

Besides the work cited above, Rodenhuis (1919) has proposed the name *Endolimax pileonucleatus* for the amoeba in question. The author's grounds for proposing this name are not clear. One thing of interest in this paper is that the author has found and described what he believes to be several stages in the division of the nucleus. In the paper in which Chauchemez (1921) describes the finding of "iodine cysts" in the faeces of pigs, he notes that Brumpt (personal communication) accepts Dobell's genus *Iodamoeba*, but because he does not believe that the amoeba can be identified with any previously described species, he (Brumpt) proposes the name *Iodamoeba wenyoni*.

MATERIAL AND METHODS.

While a number of stools containing "iodine cysts" have come to our notice during the last two years, practically all of the present work was carried out on specimens obtained from two individuals. These individuals were selected because we could obtain daily samples of faeces from them, and no objection was raised whenever we wished to administer a purgative in order to obtain the motile amoebæ. They are sisters, aged three and five years, who were born in Baltimore and have not left the state of Maryland during their life time. We have designated them Cases 1 and 2 respectively. As far as we can find out, they are very healthy children who have no history of intestinal disorder. They show a rather heavy multiple infection of intestinal protozoa, having, besides the amoeba of the "iodine cysts," *Endamoeba coli*, *Trichomonas hominis*, and *Giardia intestinalis*. Besides these entozoa with which both cases are infected, Case 1 is infected with *Dientamoeba fragilis*. Neither case is infected with *Endolimax nana*.

In addition to studying the organisms in the living condition, they were fixed and stained in various solutions. Most of our material was fixed in Schaudinn's sublimate-alcohol solution, to which were added varying amounts of acetic acid, and then stained either with Heidenhain's iron hæmatoxylin, followed with eosin, or with Mann's methylblue-eosin stain, as modified by Dobell. A large amount of material was, however, fixed and stained in other ways in order to study it from a comparative standpoint. Among these latter fixatives may be mentioned: Carney's acetic acid-alcohol-chloroform mixture, Fleming's chromo-acetic-osmic mixture (strong formula), Bouin's picro-formol mixture, and chromo-aceto-formalin mixture. The other stains that were used were Delafield's hæmatoxylin and Dobell's alcoholic iron-hæmatein.

It is a pleasure to acknowledge our indebtedness to our laboratory assistant, Mr. Conrad Bauer, for finding the cases described above, and for obtaining faecal specimens from them daily over a period of several months. We are also indebted to Miss Ethel Norris for the drawings which appear in this paper.

THE MOTILE AMOEBÆ.

In the present paper we will limit ourselves to a detailed description of the nucleus of the present form. There are, however, certain

other characteristics of the species which should be mentioned. According to Dobell (1919) the diameter of this amoeba, when rounded, is usually from 9 to 13 μ . He also observed larger specimens up to 17–20 μ and smaller ones of 5 μ in diameter. Kuenen and Swellengrebel (1917) give the diameter as 10–12 μ and Brug (1921) states that in his experience it is 7 to 20 μ . The maximum size which Prowazek (1912) gives for his *Entamoeba bütschlii*, which is probably the same species, is 24 μ . As Dobell (1919) points out, however, Prowazek may have mistaken a small specimen of *E. coli* for the present form. In our work we find that fixed and stained specimens of the motile amoeba, when rounded, vary in diameter from 9 to 14 μ , the most usual diameter being 11 μ . The largest specimen which we have seen was 20 by 15 μ . In this connection it is interesting to note that, in our experience, the active amoebae are on the average about 2 μ greater in diameter than the cysts. This indicates that the amoeba undergoes a reduction in size just before encystment. Such a process is known to occur in the other intestinal amoebae, but Dobell (1919) maintains that it is not the case in the amoeba of the "iodine cysts."

When the motile amoebae are examined in the living condition on a warm stage, their movement is very similar to that of a small *E. coli*. The rapidity with which different specimens move varies greatly, some being very sluggish and others moving with great rapidity. Text-figure 1 represents a series of camera lucida outlines of a rapidly moving specimen drawn at intervals of 15 seconds. In such specimens there is usually no sharp distinction between ectoplasm and endoplasm. Occasionally, however, a specimen will stop its progressive movement and send out a pseudopodium which is sharply marked off from the granular body of the animal. These clear hyaline pseudopodia are formed very rarely, and we have never seen them used as organs of locomotion. We believe that they represent an abnormal condition, and that the formation of the *coli*-type of pseudopodium is the normal method of progression. In contrast to such active specimens as the one shown in Text-fig. 1, we have often observed specimens for as much as 15 minutes which constantly threw out pseudopodia and yet scarcely moved from their original position. The visibility of the nucleus in the living amoebae depends largely on the amount of the food inclusions. Specimens are often seen which contain numerous bacteria and almost every type of intestinal debris, as in the specimen shown in Fig. 1, Plate VIII. Such specimens, when alive, remind

one of *E. coli*, but the nucleus is rarely visible in them. In other specimens which contain little or no food, the nucleus with its large central karyosome can often be clearly seen.

TEXT-FIG. 1. Series of camera lucida outlines of a very active specimen of *Iodamoeba williamsii* which shows the characteristic changes in shape in such specimens. The specimen was observed on a warm stage, and the outlines were made at 15 second intervals. Numbers 1-15 indicate the shape and position of the amoeba at each consecutive interval.

The size of the nucleus varies with that of the amoeba, being roughly about one quarter the diameter of the entire specimen. Its structure can be considered under four heads: (1) the nuclear membrane, (2) the karyosome, (3) the peripheral layer of granules ("peripheral chromatin"), (4) the linin threads which extend from the karyosome to the nuclear membrane. The nuclear membrane is clearly visible in any of the stains used and often contains granules or even small lumps of deeply staining substance. Whether or not these masses are chromatin cannot be said at the present time. The large karyosome is, in our experience, always located centrally in the nuclei of the motile forms. Its diameter is roughly about one half that of the nucleus (see Plate VIII, Figs. 1-4). When stained with

haematoxylin stains, it often is not homogeneous in structure, but exhibits a lighter central area. When stained with Mann's stain, there are often present one or several darker staining granules in the center. Figure 3, Plate VIII, shows a specimen which contains three of these structures. The nature of these structures is not clear—certainly there is no valid reason for assuming them to be centrioles, as is done by Prowazek (1912) in his description of "*Entamoeba bütschlii*," which is probably the same form. Immediately surrounding the karyosome, there is a layer of granules which we have termed the peripheral granules and which is often termed "peripheral chromatin." In a large number of the specimens stained with either iron haematoxylin and eosin, or with Mann's stain, this layer of peripheral granules appears as a simple layer divided into segments by numerous radiating septa (Plate VIII, Figs. 1 and 2). In well-stained specimens, however, it can be seen that this is not the true structure of this layer, but that each segment is in reality an ovoid granule embedded in a deeper staining matrix. (Plate VIII, Figs. 3 and 4.) In the most favorable specimens, with careful focusing of the microscope it is even possible to see that each of these granules has a slightly higher refractive index than the surrounding protoplasm. This layer of peripheral granules is sharply differentiated from the karyosome, in material stained in Mann's stain—the layer of granules staining blue and the karyosome red. The appearance in iron haematoxylin and eosin depends largely on the extraction of the haematoxylin. As pointed out by Dobell (1919) the granules take both nuclear and plasma stains, but give up the former much more readily than the karyosome. Not only is this true in our experience, but we have also found that the matrix in which the granules are embedded is intermediate in this respect—decolorizing much sooner than the karyosome, but retaining the stain (haematoxylin) longer than the actual granules. It is, therefore, possible to stain the entire granule layer black like the karyosome, or to decolorize the nucleus until the actual granules are colorless, but the matrix around them is still dark (Plate VIII, Fig. 3), or to decolorize the nucleus to such an extent that the entire granule layer—including both the actual granules and their surrounding matrix—appears practically colorless, with the karyosome still retaining an almost black stain. In the latter case the entire granule layer can be retained with eosin. While the above description of the way in which the granules behave with haematoxylin stains is true in general, one finds that each granule may show some

idiosyncrasy in the way in which it retains the stain. Such a condition of affairs is shown in the nucleus of the specimen in Text-fig. 2. Here some of the granules have completely given up the stain, while others cannot be distinguished from the matrix in which they are embedded. We shall see later that this variability with which the different granules take or give up haematoxylin probably explains some of the peculiar pictures which are often seen in the nuclei of cysts.

TEXT-FIG. 2. Camera lucida drawing of a motile specimen of *I. williamsii*, stained with iron-haematoxylin. Note the peculiar way in which some of the peripheral granules around the karyosome are stained while others are not. Compare with Figs. 1-4, plate VIII. $\times 4,300$.

In the majority of our specimens fixed with Schaudinn's fluid and stained with either iron haematoxylin and eosin or with Mann's stain, it is possible to distinguish several fine linin strands running from the karyosome to the nuclear membrane. These linin fibers must not be confused with the appearance of septa in the granule layer noted above. These linin fibers extend through the granule layer and actually touch the nuclear membrane (Plate VIII, Figs. 1-4). Dobell (1919) does not mention these strands, but they are noted by Brug (1921). It is very difficult, however, to interpret Brug's figures. We are unable from a study of his figures of the motile form to decide whether he has decolorized the layer of peripheral granules to such an extent that a few "septa" are left, or whether he has overstained them so that the layer of granules is not differentiated from the karyosome, in which case the strands shown would be the linin strands in question. Judging from the comparative size of the karyosome to the entire nucleus, we feel that the latter is probably the true explanation of his figures.

differences between the foregoing account and the figures given by Brug (1921) are to be explained by an overstaining of his specimens with haematoxylin. In such overstained specimens, the layer of peripheral granules cannot be differentiated from the karyosome, and this gives the nucleus the appearance of containing a large central karyosome, connected to the nuclear membrane by a few linin strands.

THE CYST.

It is difficult to give a single measurement of size for the cysts, due to the fact that so many of them are irregular in shape. To overcome this difficulty in the case of the irregular cysts, Dobell (1919) has taken the average of the greatest length and the greatest width, as the "size." In using this measure of "size" which we have found very valuable, we have found that most cysts lie between $6.4\ \mu$ and $16.6\ \mu$, and that the average is about $9.1\ \mu$. In our experience, the irregularity in shape of the cysts varies considerably from day to day in the same infection. Some days almost all of the cysts are nearly spherical, and on other days many are very irregular in shape. In this connection mention should be made of a most remarkable structure which we found on one of our prepared slides, which is undoubtedly an "iodine cyst." A camera lucida outline of this cyst is given in Text-fig. 3 *B*. By comparing this cyst with the one beside it, which is of ordinary size, it can be seen that it is abnormally large and irregular in outline. This monster contained four glycogen vacuoles. In spite of its abnormalities, however, the structure of the nucleus leaves no doubt that it is a true "iodine cyst." In our work we have not found any other cysts which were comparable to this one, either in size or in shape.

It is not necessary to describe the appearance of the cysts, when examined in the living state, or in iodine solution, as this has been done by a number of investigators. We can, therefore, pass directly to a consideration of the nuclei of the cysts. The mature cysts practically all contain a single nucleus. Occasionally supernucleate cysts, with two nuclei, are found, but these are rare. In order to ascertain approximately the percentage of the cysts that are binucleate, we examined 2,000 cysts consecutively, as they appeared on some of our prepared slides. Among those 2,000 cysts only four contained two nuclei—giving a percentage of 0.2. As far as we can find, no investigator has described cysts with more than two nuclei.

With the exception of the presence of the fine linin fibers extending

from the karyosome through the layer of peripheral granules to the nuclear membrane, the foregoing account agrees, almost in detail, with the description given by Dobell (1919). We believe further that the

The nucleus of the cyst possesses the same structures which were described for that of the motile form, viz., (1) a nuclear membrane, (2) a karyosome, (3) peripheral granules ("peripheral chromatin"), and (4) linin strands running from the karyosome to the nuclear membrane. Structures (1) and (4) are the same as in the motile amoebae, but the position and relationship of (2) and (3) are greatly

TEXT-FIG. 3. A, typical cyst of *I. williamsii* with a single glycogen vacuole, v. B, unusually large and irregular cyst containing four glycogen vacuoles, v.

modified, when the amoeba encysts. These changes can readily be seen by comparing the nuclei of the motile forms, shown in plate VIII (Figs. 1-4), with those of the cysts, shown in the same plate (Figs. 5 and 6). The karyosome, instead of being central, as it is in the motile amoeba, becomes eccentrically placed, and the layer of granules, instead of surrounding the karyosome, becomes massed to one side of it. The number of these granules varies greatly. Sometimes they are several layers deep and completely fill the nuclear cavity on one side of the karyosome, and at other times, they are comparatively few in number and are closely applied to one side of the karyosome (Plate VIII, Figs. 5 and 6). Of course the actual appearance of the nucleus

varies according to the pole from which it is viewed. If it is seen from the pole toward which the granules are displaced, the contour of the karyosome is obscured, giving a picture like the one shown in Plate VIII, Fig. 7. If, on the other hand, it is seen from the opposite pole, i.e., the pole toward which the karyosome is displaced, the latter is seen surrounded by the granules (Plate VIII, Fig. 8). In comparing these two figures (Plate VIII, Figs. 7 and 8), it must be kept in mind that they differ markedly in another respect, viz., the number of granules present in each.

As in the case of the motile amoebae, our description of the structure of the nucleus of the cysts agrees, almost in detail, with that of Dobell (1919). Dobell, however, does not note or figure the linin strands, running from the karyosome to the nuclear membrane, which are shown in figure 6, and, to a less extent, in Fig. 5, Plate VIII. Most of our material, which was fixed in Schaudinn's fluid and stained in either iron haematoxylin and eosin or in Mann's stain, showed these strands. They were, however, absent in some of the cysts, as is shown in Text-fig. 3 A. Incidentally this figure is very similar to some of those given by the above investigator.

While our results are in accord with those of Dobell, they are not in agreement with those of Brug (1919 and 1921), Nöller (1921), or Kofoed, Kornhauser, and Swezy (1919). Let us first consider the findings of Brug. In his earlier paper (1919) he describes the nucleus as containing a large karyosome and a crescent-shaped mass of chromatin granules which is separated from the karyosome by a considerable space. This description is repeated in his later paper (1921). It is very difficult to ascertain the exact nature of this crescent-shaped mass of chromatin granules from his description. One would be led to think that the author means that the crescent-shaped mass is composed of chromatin of the same nature as found in the karyosome. Certainly, there is no evidence that he means anything like the peripheral granules which we have just described, and which show an entirely different staining reaction from the karyosome when stained with Mann's stain. This interpretation is further supported by the fact that the author places the amoeba in the genus *Endolimax*—presumably on his interpretation of the structure of the nucleus. Another thing of interest is that the author apparently believes that the crescent-shaped mass of chromatin granules arises only after the amoeba encysts, whereas the peripheral granules, which we have just described, were represented in both the free forms and in the cysts.

We were, at first, unable to explain Brug's figures. Later on in our work, however, we obtained preparations which appear so similar to his that we feel that we have an explanation of his findings. In some of our material which was fixed with Schaudinn's fluid and stained with iron haematoxylin, we obtained many specimens similar to those shown in Plate IX, Figs. 9 and 10. These specimens show the presence of a crescent-shaped mass widely separated from the karyosome, and are almost identical with some of Brug's figures. One thing is at once apparent, however, viz., the crescent-shaped mass is composed of the same large granules (" peripheral chromatin ") which Dobell and the present authors have described, as occurring between the karyosome and the nuclear membrane. The mass itself often stains as black as the karyosome, due to the way in which the matrix around the granules is stained. Why, then, should this mass of granules be so widely separated from the karyosome in this material, and not in the material described above? We first thought that it was because of shrinkage due to improper fixation. This is hardly the case, however, because in another set of the same slides, fixed in the same manner at the same time, but stained in Mann's stain, we could trace the granules clear up and in contact with the karyosome. It seems more probable, therefore, that the appearance of the crescent-shaped mass is to be explained on the assumption that the layer of granules next to the karyosome often gives up the haematoxylin stain more readily than the remaining granules. This assumption is supported by the fact that in haematoxylin specimens one can often see the granules extending to the karyosome, but those nearest to the karyosome are much more lightly stained than those further away. Another fact may enter into the explanation, and that is the question of the matrix in which the granules are embedded. This matrix undoubtedly takes the haematoxylin more readily than the actual granules, and the appearance of the separate mass may be due in some part to the presence of less matrix near the karyosome than further away. At any rate it is interesting to be able to duplicate Brug's results and see that his crescent-shaped mass is composed of the same peripheral granules which we described above. Furthermore, we feel that it is probable that in such specimens, as are shown in Plate IX, Figs. 9 and 10, there is a layer of unstained granules between the karyosome and the dark crescent-shaped mass.

While Nöller (1921) does not give a detailed description of the nucleus, it is clear that he agrees with the description given by Brug.

He states in a footnote (page 41) that the characteristic nuclear structure can best be seen in Delafield's haematoxylin or in haemalum preparations, and that the half-moon (crescent-shaped mass) can be seen only poorly in iron-haematoxylin preparations. He also states that not a single one of Dobell's figures depicts the true nuclear structure of the cysts, due to the fact that differentiation has been carried too far. In regard to Nöller's first statement we can say that we have been able to get this structure in iron haematoxylin as well as in Delafield's haematoxylin. We also believe that the second statement is based on a misinterpretation of the facts. It implies that the crescent-shaped mass is something different from the granules described by Dobell. As we have shown above, however, it is in reality composed of the same granules which Dobell shows in his figures, and which we have illustrated in Plate VIII, Figs. 5 and 6. Furthermore, we feel that the mass of these granules extends to the karyosome, and that there should be no space between them and the karyosome.

Besides the appearance of the crescent-shaped mass, many other peculiar nuclear pictures are often seen in iron-haematoxylin preparations. Some of these suggest very strongly the condition of affairs often met with in *E. nana*, and may offer an explanation of the results obtained by Kofoid, Kornhauser and Swezy (1919)—especially as these investigators used this stain almost exclusively. A few of these nuclei are shown in Plate IX, Figs. 11 to 16. In the case of the nucleus shown in Fig. 11, the mass of granules has been so poorly stained that the contour of none of them can be seen. Incidentally it may be remarked that this nucleus is viewed obliquely from the pole toward which the karyosome is displaced. In Figs. 12 and 14 a few of the granules themselves have apparently taken the haematoxylin stain. In Fig. 15 practically all trace of the mass of granules has been removed by destaining. The nucleus in Fig. 16 is very similar to that of *E. nana*. Here it seems probable that the larger black mass is the karyosome and the smaller one with the strand which attaches it to the larger mass represents the mass of granules. We do not know why some granules in the mass should decolorize so readily and others retain the stain, but we do know that the different granules often show many idiosyncrasies, in the way in which they behave, in the iron-haematoxylin process. We feel that, if any of these cysts had been stained in Mann's stain, we would have been able to demonstrate the karyosome and the peripheral

granules, the former staining red and the latter blue, because some of the identical material, fixed at the same time, was stained in Mann's stain and did give these results. The above statements must not be construed to mean that accurate pictures of the nucleus are *never* obtained with iron haematoxylin. This stain, especially if used with eosin, often gives beautiful results, but it also often behaves as described above.

Our suggestion that the results of Kofoed, Kornhauser and Swezy can be explained in the above manner is not based on work with the "iodine cysts" alone. We have also examined a large number of both motile and encysted forms of *E. nana*. This was done to see if, with our technique, we could demonstrate the same type of nucleus in *E. nana* as we did in the "iodine cysts." In none of these specimens, however, have we been able to demonstrate anything comparable to the peripheral granules described above. The karyosome was always represented by one or several masses which were generally connected by a fine chromatic strand. A typical cyst of *E. nana* is shown in Plate IX, Fig. 17. All of our work, therefore, points to the conclusion that not only is the amoeba of the "iodine cysts" not identical with *E. nana*, but its nucleus is so different from that of the latter species, which is the type species of the genus *Endolimax*, that it cannot even be placed in the same genus.

Nomenclature*

The nomenclature of the parasitic amoebae is somewhat confused at the present time. The correct parent genus is undoubtedly *Endamoeba* Leidy 1879, type by original designation *E. blattae*. Since the formation of this genus the parasitic amoebae have been divided into a number of genera, the distinguishing characteristics of which are chiefly differences in nuclear structure (see Dobell and O'Connor, 1921). If we accept this classification the amoeba of the "iodine cysts" cannot be placed in the genus *Endamoeba* Leidy 1879, type *E. blattae* (*Entamoeba* Casagrandi and Barbagallo, 1895, type *E. coli*), or *Endolimax* Kuenen and Swellengrebel 1917, type *E. nana*. We believe that Brug's contention that the amoeba is a species of *Endolimax* rests on a misinterpretation of the nature of the crescent-shaped mass. If this mass were composed of the same type of chromatin as the

* We arrived at the conclusions expressed in this section, particularly those in regard to the specific name, after a conference with Dr. C. W. Stiles. It is a great pleasure to express our indebtedness to Dr. Stiles for his helpful advice.

karyosome, the nucleus would resemble an *Endolimax* nucleus. This is not the case, however, because the crescent-shaped mass is, in reality, an aggregation of the same type of large granules which surround the karyosome in the motile stage. While these granules may be a kind of chromatin, they do not give the same staining reaction as the karyosome, and are probably of an entirely different nature. The contention of Kofoid, Kornhauser and Swezy that the "iodine cysts" are the uninucleate cysts of *E. nana* has already been considered. As the amoeba cannot be placed in the genus *Endolimax*, the first available name is *Iodamoeba* of Dobell (1919). Kuenen and Swellengrebel's (1917) name, "*Pseudolimax*," was proposed, distinctly as not subject to the rules of nomenclature, and hence not as a true generic name.

Nöller (1921) and Brug (1921) have both examined Prowazek's original slides and agree that *Entamoeba williamsi* Prowazek 1911 is a mixture of *E. coli* and the amoeba of the "iodine cysts." Practically all observers agree that the species which Prowazek described the next year under the name *Entamoeba bütschlii* is entirely the amoeba of the "iodine cysts." The question arises, then, as to which name should be used for the amoeba of the "iodine cysts." If *williamsi* Prowazek, 1911, is to be considered a synonym of *E. coli*, *bütschlii* Prowazek, 1912, is the first available name. If, however, it can be shown that *williamsi* Prowazek, 1911, is available for the present form, it must take precedence over *bütschlii* Prowazek, 1912.

Castellani and Chalmers, 1913, and Hartmann, 1913, were the first authors to consider *E. williamsi* Prowazek. The former investigators considered it a probable synonym of *E. coli* but the latter clearly indicated that he considered it different from *E. coli* because he designated it *Entamoeba coli* var. *williamsi*. We have, therefore, two revisors of the same year for Prowazek's species and there is no way of fixing precedence by any dates given in the publications. We are, however, justified in accepting Hartmann's revision because he *definitely* stated that he considered *williamsi* different from *coli* whereas Castellani and Chalmers (1913, p. 269) gave *williamsi* as a *probable* synonym of *coli*. Another fact which necessitates our acceptance of Hartmann's revision is that the first subsequent investigator to recognize the nature of the organism under question (Brug 1919) and to designate the type material (Brug 1921) followed the spirit of Hartmann's revision. It is very important to note that Hartmann based his distinction between *coli* and *williamsi* on the

motile amoeba and, therefore, if it can be shown that the motile stage of *williamsii* is different from *E. coli* we will have to retain the name *williamsii* for this new species, and it will not be permissible to consider it a synonym of *E. coli*. Not only have Nöller (1921) and Brug (1921) shown that Prowazek's original slides contained both *E. coli* and the amoeba of the "iodine cysts," but an examination of Prowazek's description of *E. williamsii* shows that the motile stages of this form (Figs. 1 and 2) are those of the "iodine cysts." A further fact of paramount importance is that Brug (1921) clearly designated Prowazek's original slides as the type material of the species. The revision of Prowazek's *williamsii* by Hartmann (1913), the designation of Prowazek's original material as the type material of *williamsii* by Brug (1921), and the identification of this material with the amoeba of the "iodine cysts" by Brug (1921) and Nöller (1921), make it quite clear to us that under the International Rules of Nomenclature (Articles 29, 30, and 31) *williamsii* is the valid name of the species in question.

To sum up, then, we feel that the name of the amoeba of the "iodine cysts" should be *Iodamoeba williamsii* Prowazek 1911.

CONCLUSIONS.

1. In the nucleus of the motile amoeba of the "iodine cysts" there is a well-defined nuclear membrane, a large central karyosome, a single layer of peripheral granules ("peripheral chromatin") which surrounds the karyosome, and a few fine linin strands which run from the karyosome through the layer of peripheral granules to the nuclear membrane. The peripheral granules show a different staining reaction from the karyosome.

2. The nucleus of the cysts contains the same structures as that of the motile amoebae. The karyosome, however, becomes eccentrically placed, and the peripheral granules, which vary greatly in number among different nuclei, become localized on one side of the karyosome, between it and the nuclear membrane.

3. With the exception of the linin strands, our description agrees with that of Dobell. The crescent-shaped mass which is described by Brug and Nöller in the nuclei of the cysts, is composed of the granules noted above. In cysts which are stained with iron haematoxylin and decolorized to too great an extent we have found many pictures which remind one very much of *E. nana*, and may explain the findings of Kofoid, Kornhauser and Swezy.

4. The structure of the nucleus of the amoeba of the "iodine cysts" makes it impossible to identify it with *Endolimax nana* or even to include it in the genus *Endolimax*.

5. The name of the amoeba of the "iodine cysts" should be *Iodamoeba williamsi* Prowazek 1911.

BIBLIOGRAPHY.

- BRUG, S. L.
1919. *Endolimax Williamsi*: the amoeboid form of the iodine-cysts. Indian Journ. Med. Res., 6, 386.
- BRUG, S. L.
1921. Die Jodzysten. Arch. f. Schiffs- u. Tropenhyg., 25, 47.
- CASTELLANI, A., AND CHALMERS, A. J.
1913. Manual of tropical medicine. 2d Edition (New York).
- CHAUCHEMEZ, L.
1921. Fréquence des amibes iodophiles chez le porc, en France. Bull. Soc. Path. Exot., 14, 321.
- DOBELL, C.
1919. The amoebae living in man: a zoological monograph. (London.)
- DOBELL, C., AND O'CONNOR, F. W.
1921. The intestinal protozoa of man. (London.)
- FLU, P. C.
1918. Onderzoekingen over protozoën uit den menschelijken darm. Geneesk. Tijdschr. v. Nederl.-Indië, 58, 85.
- HARTMANN, M.
1913. Morphologie und Systematik der Amöben. Kolle u. Wassermann's Handb. d. path. Mikroorg., 2 Aufl., 7, 607.
- KOFOD, C. A., KOENHAUSER, S. I., AND SWEZY, O.
1919. Structure and systematic relationships of the "iodine cysts" from human feces. Milft. Surg., 45, 30.
- KOFOD, C. A., AND SWEZY, O.
1921. On the free, encysted, and budding stages of Councilmania laffeyi, etc. Univ. California Pub. Zool., 20, 169.
- KUENEN, W. A., AND SWELLENGREBEL, N. H.
1917. Korte beschrijving van enkele minder bekende protozoën uit den menschelijken darm. Geneesk. Tijdschr. v. Nederl.-Indië, 57, 496.
- MATTHEWS, J. R.
1918. Observations on the cysts of the common intestinal Protozoa of Man. Ann. Trop. Med. and Parasitol., 12, 17.
- NÖLLER, W.
1921. Ueber einige wenig bekannte Darmprotozoen des Menschen und ihre nächsten Verwandten. Arch. f. Schiffs- u. Tropenhyg., 25, 35.
- O'CONNOR, F. W.
1920. A preliminary note on two intestinal parasites in pigs. Med. Journ. Australia, 2, 337.
- PROWAZEK, S. v.
1911. Beitrag zur Entamoeba-Frage. Arch. f. Protistenk., 22, 345.

PLATE VIII.

PLATE IX.

- PROWAZEK, S. v.
1912. Weiterer Beitrag zur Kenntnis der Entamoeben. *Ibid.*, *26*, 241.
- RODENHUIS, Y.
1919. Bijdrage tot de kennis van die jooceysten en hunne amoeben. *Geneesk. Tijdschr. v. Nederl.-Indië*, *59*, 813.
- WENYON, C. M.
1915. Observations on the common intestinal protozoa of man: their diagnosis and pathogenicity. *Journ. Roy. Army Med. Corps*, *25*, 600.
- WENYON, C. M.
1916. The protozoological findings in 556 cases of intestinal disorder from the Eastern Mediterranean War Area. *Ibid.*, *26*, 445.
- WENYON, C. M., AND O'CONNOR, F. W.
1917. An inquiry into some problems affecting the spread and incidence of intestinal protozoal infections, etc. *Ibid.*, *28*, Nos. 1-6. Reprinted, with additions, as *Human Intestinal Protozoa in the Near East* (London).

EXPLANATION OF PLATES.

All figures are camera lucida drawings of specimens fixed with sublimate-alcohol (with or without acetic acid), and stained as indicated. With the exception of Fig. 17, which is a cyst of *Endolimax nana*, all figures are drawings of *Iodamoeba williamsi*. Magnification $\times 4,300$.

PLATE VIII.

FIGS. 1-2. Active amoebae stained with iron haematoxylin and eosin. The peripheral granules of the nucleus appear as a segmented ring surrounding the karyosome.

FIG. 3. Precystic amoeba stained with Mann's methylblue-eosin mixture. The peripheral granules surrounding the karyosome show very distinctly.

FIG. 4. Active amoeba stained as above. Note the linen strands running from the karyosome to the nuclear membrane.

FIGS. 5-6. Cysts stained with Mann's methylblue-eosin mixture. Note the change in position of the karyosome and the peripheral granules. The glycogen masses have been dissolved, leaving the clear vacuoles shown in the cytoplasm.

FIG. 7. Nucleus of cyst, stained as above, but viewed from the pole toward which the peripheral granules are displaced.

FIG. 8. Nucleus of cyst, stained as above, but viewed from the pole toward which the karyosome is displaced.

PLATE IX.

With the exception of Fig. 17, which is a cyst of *E. nana*, the drawings in this plate show incorrect nuclear pictures due to faulty staining. Magnification $\times 4,300$.

FIGS. 9-10. Cysts stained with iron-haematoxylin which show the presence of the "crescent-shaped mass" in the nucleus. This "crescent-shaped mass" is composed of the same peripheral granules as are shown in Figs. 5 and 6.

FIGS. 11-16. Nuclei of cysts stained with iron-haematoxylin. These peculiar nuclear pictures are due to the way in which the mass of peripheral granules often behave in this stain. These figures are discussed fully in the text.

FIG. 17. Cyst of *Endolimax nana* stained with Mann's methylblue-eosin mixture. Compare with cysts shown in Figs. 5 and 6, and note the peculiar karyosome and absence of "peripheral chromatin" in this species.