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Notes on Councilmania lafleuri (Kofoid-Swezy 1921).

By TOYNBEE WIGHT. (Introduced by L. B. Becking).

In June, 1921, Kofoid and Swezy announced a new species of intestinal parasite to which they gave the name of *Councilmania lafleuri.* They attributed to their discoverey certain peculiarities found in no other intestinal amœba, which if confirmed, would prove they were dealing with an entirely new species. They also suggested certain pathogenic properties. A somewhat strenuous denial of the accuracy of their findings was made by certain European protozoologists. By and large, in this country at any rate, *Councilmania lafleuri* has been accepted as a biologic fact.

With abundant material from patients from all over the world, we began an immediate search for the new amœba. It is the conclusions drawn from the examination of many hundreds of stools and carefully stained preparations of specimens containing cysts and free forms that form the subject of this paper.

For some time previous to the announcement of Kofoid's new amœba we had been doubtful of the accuracy of Dobell's statement that "the sudden extrusion of clear blade-like pseudopodia —so often seen in *E. histolytica*—is never observed in *E. coli.*" (Amœba Living in Man, 1919, p. 78.) As this doubt was in line with Kofoid and Swezy's contention, we carefully followed their method of fixation, *viz.*, hot Schaudinn at 60° C., and observed results.

Since the nearest morphological neighbor to *Councilmania* is *E. coli* let us give Kofoid's own comparison of the two organisms:

FREE STAGES.

Councilmania.	*E. coli.*
(1) Very active—pseudopodia thrust out suddenly—ectoplasm sharply differentiated.	(1) Sluggish—ectoplasm not sharply differentiated.
(2) Red cells ingested readily.	(2) Red cells not ingested normally.
(3) Peripheral chromatin thin layer. Karyosome large eccentric with halo—often seen in premitosis.	(3) Peripheral chromatin thicker. Karyosome small spherical with halo. Generally eccentric.

It will be observed that the above are differences in degree only, and insufficient in themselves on which to base a ready distinction. With regard to item No. 2, an examination of thousands of free amœbæ of the *Councilmania* type during the last few years has failed to show *one* which contained a red cell. We have tried feeding active forms with fresh blood cells and then examining, but without result. The diet of these amœbæ—as Kofoid himself shows—is largely bacterial in character. Frequently larger food particles—such as cysts of Chilomastix, when these are also present—may be seen included. It would appear from the dietary evidence that *Councilmania* is a fæcal scavenger, and in no sense a true parasite. That it may encircle blood cells occasionally, if free blood be present in the gut, is not improbable, but such unusual diet does not make of it a tissue parasite.

Let us now turn to the more important cystic differences as given by Kofoid and Swezy:

ENCYSTED STAGES.

Councilmania.	*E. coli.*
(1) Cyst wall very thick.	(1) Cyst wall thin.
(2) Spheroidal, ellipsoidal or asymmetrical—less often spherical.	(2) Generally spherical.
(3) Less readily stained.	(3) More readily stained.
(4) Glycogen body resistant to Iodine.	(4) Glycogen body stains readily in Iodine.
(5) Nuclei with little peripheral chromatin-slightly eccentric dispersed karyosome.	(5) More peripheral chromatin. Small eccentric massed karyosome.
(6) Chromatoidal bodies less acicular in early stages—massed centrally in later stages and contributing to chromophile buds.	(6) Chromatoid bodies more distinctly acicular—less central massing—no relation to segregation of chromophile cytoplasm.
(7) Chromophile ridge forms bud through pore in cyst wall—which detaches uninucleate amœbæ.	(7) Budding unknown.

Distinctions No. 1 and No. 2 are matters of degree and generalities, but I hope to show these differences in shape and thickness of cyst wall have considerable bearing in this discussion.

Item No. 3 is a mistake. There is no especial difficulty in staining *Councilmania.* Occasionally one strikes a type that is more difficult to stain than the usual run, whether this be *E. nana, E. coli,* or *E. histolytica;* and speaking generally the *Councilmania* type are stained as easily as any other of the intestinal organisms.

Item No. 4. There is often seen a retraction of the cytoplastic contents from some part of the cyst wall. Some stools may show

a majority of the cysts with this peculiarity. There is no suggestion by chemical tests that this is a glycogen vacuole. As a matter of fact, glycogen is rarely seen in cysts of the *Councilmania* type. I attribute this particular vacuolisation to osmotic causes.

Items No. 5 and No. 6 are differences in degree only.

Item No. 7, "Chromophile ridge forms bud through pore in cyst wall which detaches uni-nucleate amœbæ"—This last is the valid basis for naming *Councilmania* as a new species. It is very easy to stain specimens in suitable material showing the ridge. Do not carry decolorization too far and stain somewhat deeply—but it must be noted that the ridge is easily decolorized, far sooner than are the chromidial bodies. By the use of a special stain which shows up the cyst wall distinctly, it may be shown that the chromidial ridge is part of the wall itself. The chromidial ridge in our preparations is not of cytoplastic origin; nor is it of the same material as the chromidial bodies, for with adequate decolorization it disappears entirely, leaving the chromatoid bodies still heavily stained. There is no connection that we can find between the chromatoid bodies and the ridge. The latter seems to be a fold in the cyst wall and its chromophilic character to partake of the nature of a shadow.

We now come to the "bud" through the "pore" in the cyst wall. Examination of many thousands of cysts in fresh saline smears has failed to show this phenomenon. It does not seem possible that Drs. Kofoid and Swezy can have seen it often, nor do they make the claim to have done so. All their illustrations of this phenomenon are from fixed, stained material. It may be suggested that if they saw this phenomenon in a fresh smear in physiological saline, abnormal physical conditions or some pathological process had brought about the result. If one adds hot Schaudinn to a concentration of cysts, and then examines a smear from the mercurial solution, oftentimes a very large proportion of the cysts will now show protruding "buds"—the "pore" varying from a minute aperture to a frankly ragged tear in the cyst wall.

We have stained many hundreds of such preparations, and reproduced each and all of the wonderful illustrations of Kofoid and Swezy. Again it should be emphasized that before such fixation no such pictures may be seen. After fixation and according to the concentration of cysts, to their degree of irregularity of contour and to the rigidity of their walls, few or many budding

cysts may be seen. Let us now remember the two first distinctive characteristics of *Councilmania* as compared with *E. coli* as given by Kofoid and Swezy, viz, greater thickness of walls and irregularity of contour of the cysts, "often ellipsoidal or asymmetrical." Now, since a non-rigid coherent body tends to assume a spherical shape in a liquid medium, it is obvious that the cyst walls of this type are of a certain rigidity. We have, therefore, favorable conditions for fracture if sufficient pressure be employed. It is suggested that the osmotic pressure of the strong mercurial solution used as fixative may be the cause of the appearances as seen in stained specimens. It is an undeniable fact that measurements of stained cysts are proportionately less than those taken of untreated cysts in a fresh saline smear. It is also true that many cysts after fixation and staining show a radiate structure of quasi-crystalline appearance. The unpublished work of John Field and Dr. L. B. Becking on "Anisotropic and Isotropic Agar" suggests an interpretation of these radiating striæ. Their appearance indicates a condition of stress in the direction of the radius of the cyst. The pressure within and without the cyst must be very great. If a break occurs at a weak point it must be in the direction of one of these radial lines of stress. This internal stress may account for the "bud."

If "budding" were a real and not an artificial process one would expect to find detached amœbulæ. We have never seen these even in those specimens apparently loaded with "budding" forms. The mass of extruded material lies around the neighborhood of the break, and is always in physical union with the cytoplastic body of the cyst. It is true that we have never made out more than one nucleus in an extrusion—when nuclei are present at all—but there is occasionally seen another nucleus in the act of passing through the "pore" whilst the mass of the preceding cytoplastic extrusion is still in union with the body of the cyst. Many extrusions—probably the majority—contain no nucleus at all.

In this connection it may be remembered that a few months ago at a meeting of this society there was a beautiful demonstration showing how certain chemical irritants caused an extrusion of the nuclei in pigeon's blood. Frequently one may see the nucleus of a free amœba entirely cast out after hot fixation.

CONCLUSIONS.

It is suggested that there are at least two types of *E. coli;* one—of which a perfect description is given by Kofoid under the name of *Councilmania*—characterized by possessing clear blade-like pseudopodia, well differentiated from the endoplasm, occasionally of exceptional motility, which may be retained even after prolonged centrifugation in cold tap water in the process of concentration. This type seems to have a tendency to the formation of irregularly contoured, thick-walled cysts. Such cysts as these are easily fractured by immersion in a hot mercurial solution such as Schaudinn.

The other type is more sluggish, and has not the clear blade-like pseudopodia in the free state. By concentrating a sufficient number of the cysts of this type and subsequently fixing in hot Schaudinn, some at least of the cysts will probably give the appearances to which Kofoid and Swezy have applied the name *Councilmania.* Whilst Kofoid's *Councilmania* would seem to refer to the first type mentioned, if my reasoning be correct, there is either no such species as *Councilmania* or there is no such organism as *E. coli.* For all organisms that we have been accustomed to include under the term *E. coli* are potential *Councilmania* on the only real distinction made between the two organisms, viz, budding—and this latter phenomenon depends on purely physical attributes and artificial outside causes.

Condition of internal stress produced by osmosis in combination with a rigidity of the cyst wall may be the necessary factors in producing "buds." Whatever be the cause, "budding" forms are not seen in the fresh specimen, or, if they should be, may be ascribed to a pathologic or abnormal physical condition.

Amœbæ of the type described as *Councilmania* are exceedingly common. There is no evidence that they have pathogenic qualities. Their normal diet would class them as fæcal scavengers.

In a stool from which stained specimens of "budding" cysts may be easily made, it is impossible to find any detached "amœbulæ"—which leads one to infer that "budding" is produced artificially in the process of fixation and staining. Many "buds" may be found in direct smears from concentrated cysts after fixation in hot Schaudinn, though none at all are to be seen in fresh saline smears from the same original material.

Budding of amœbulæ in the bowel of the host is contrary to natural reason. It would be suicidal and is therefore impossible,

except as a pathological process. That some such process takes place after ingestion of the cysts by a new host is, of course, possible or even probable.

If "budding" be not a normal process within the bowel, there is no excuse for the naming of a new species. On the other hand, if "budding" be a natural process *E. coli* must be the rarest of intestinal inhabitants and *Councilmania* the commonest.

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Revised formula for the rainbow medium.

By TOYNBEE WIGHT. (Introduced by L. B. Becking).

Since I introduced the Rainbow Medium some three years ago for the study of chemical reactions in the bacterial flora of the gastro-intestinal tract, it has been found possible to simplify the technique of its manufacture to a fool-proof stage.

The formula I now use is:

Distilled water	1000 cc.
Agar	20 gm.
Liebig meat extract	5 gm.
Peptone (Difco)	20 gm.
Normal soda	3 cc.

Boil till agar all dissolved. Add indicators. (Andrade 10 cc. Saturated aqueous Brom-thymol-blue 40 cc. Saturated aqueous Phenol-sulphone-phthalein 20 cc.—The last indicator may be omitted, though if this be done, the higher degrees of alkaline formation may not be readable).

Filter through a sheet of absorbent cotton repeatedly until the temperature of the filtrate has fallen to 55C. Stir filtrate rapidly whilst adding one gram of Basic Lead acetate in 100 cc. of warm distilled water. Simultaneously add in the correct proportions whatever sugars it is desired to use. Repeat filtration through the cotton two or three times. Fill the tubes to the desired depth. Then plug. Sterilise at 10-15 lbs. for twenty minutes. Make long slants—not short as in double Russell tubes. Stab centrally with needle and stroke surface. (Kligler, for a similar medium, advises stabbing close to the side of the tube at the lower edge of the slant, but this method does not give as much information as the central stab).