A NEW NON-PATHOGENIC TETRAGENOUS AMEBA. I.

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PLATES 44 AND 45.

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In the study of the amebae of dysentery we have discovered in the feces of healthy persons a new type of ameba, which, like the dysentery ameba, forms a cyst containing four daughter nuclei, but which differs from it in biological and morphological properties. Experiments made upon animals and clinical findings have demonstrated the non-pathogenicity of the new organism.

The ameba to be described has been observed by the author in six cases; i.e., in five persons with a normal condition of the intestines and in one having a chronic catarrh of the large intestine, not dysenteric in character. In every instance the vegetative form of the ameba was found in the saline (Carlsbad salt) diarrheal stools and the cyst in the formed stools. The persons failed to show any symptoms of amebic dysentery, even after long purging; on discontinuing purgation they evacuated normal dejecta and showed no manifestations of illness.

Morphological.

As fresh stools as possible were used for the study. The vegetative forms were examined microscopically directly after defecation, in a hanging drop preparation, at a temperature of 30-37°C. Sublimate alcohol and iron hematoxylin were used for fixation and staining.

Like the dysenteric ameba, these organisms occur in three forms: (1) large vegetative form; (2) small vegetative form; (3) cyst.

Large Vegetative Form.—This form is consistently present in every part of the naturally evacuated diarrheal feces or those obtained after purgation. The active organisms can be observed readily in a hanging drop preparation from a fluid or watery stool. Their move-
ment resembles that of the dysenteric ameba, although it is on the whole slower. The changes in the shape of the organisms and the plasmic flow proceed rather slowly. One has the impression of a snail dragging its shell. The ectoplasm and endoplasm are not easily distinguished when the organism is at rest, but can be observed during movement, though not so distinctly as in *Entamoeba tetragena* Viereck. A few hours after defecation this movement is discontinued. The amebae have then become spherical in shape, and rarely extend pseudopodia. But if a preparation of this kind is returned to the thermostat, a relatively lively motion may be observed after a few hours.

The cytoplasm is, as a rule, coarsely meshed and of a tough, compact structure. It has slightly refractile properties. No blood corpuscles are found in the cytoplasm, but various bacteria or food remnants may be present. Food vacuoles can be plainly seen, but no contractile vacuoles. In the food vacuoles one frequently finds numerous bacteria, particularly staphylococci, which show vigorous molecular movement. This circumstance, observed rarely in the dysentery ameba, is present in *Entamoeba coli*. In accordance with the view of Hartmann that the kind of food remains found in the protoplasm may be considered a characteristic in differentiating the non-pathogenic from the pathogenic amebae, we desire to advance the finding of cocci as a characteristic of the ameba under discussion. The ectoplasm is of glassy transparency, light-refracting, but not as markedly so as in the case of the dysentery ameba.

The nucleus resembles a vesicular sphere and lies eccentrically. It is plainly visible even when the organism is at rest. The nuclear membrane is thick and tough, though occasionally it is smooth and thin as in the dysentery ameba. Surrounding the nucleus a narrow, light area may be observed. In this respect the nucleus resembles that of *Entamoeba coli*. As in the latter, the nucleus also contains numerous, markedly refractile, fine granules, particularly along the wall. The nuclear membrane may be absent, in which case a chain of the refractile granules forms a ring about the nucleus. In the flow of the endoplasm the nucleus always retains its globular form.

In the stained preparations the cytoplasm is not homogenous but is in part coarsely meshed, with a structure resembling that of *Enta-
*meeba coli* or the smaller form of dysentery ameba. The cytoplasm
dominantly contains brownish food residues and darkly stained bac-
teria. When the latter are located within the food vacuoles, they may
be mistaken for the dividing nucleus or the newly formed chromidia
of the enlarged nucleus. If the organisms are fixed and stained
while motile, one may obtain various pseudopodia stained light brown.
Otherwise the organism appears spherical or oval; the ectoplasm is
indistinct.

The size of the large vegetative ameba is 15 to 19μ, the largest
measuring 25μ; the nucleus is from 1.5 to 4μ. The chromatin outside
the nucleus consists of coarse granules which adhere to the nuclear
membrane. The nucleus contains abundant chromatin, which may
be distributed diversely in the fine linear alveolar tissue, in small or
in a few large particles. In the center lies a relatively large and dis-
tinct centriole as in the dysentery organism. Surrounding the cen-
triole one is often able to observe a narrow, clear area. The nucleus
does not show typical cyclic changes such as Hartmann and Werner
observed in the dysenteric ameba.

**Small Vegetative Form.**—In contrast to the dysentery ameba, it is
difficult to distinguish the small form of the non-pathogenic ameba
from the large vegetative form. The large form is found in diarrheal
stools; when, however, the feces increase in consistency the smaller
forms appear in addition to cysts. They are minute in size, measur-
ing usually 6 to 13μ, the largest found being 19μ (Figs. 7 and 8).

As in other amebae, we may regard the small form as the first stage
of the cystic transformation, even though it may persist for some
time. In this stage the food taken up by the cytoplasm has been
digested or extruded, so that there is but little foreign material
present. Some of the specimens show a few bacteria in the vacuoles
(Fig. 8). The cytoplasm is coarsely meshed. The ectoplasm is
clear and transparent, and recognizable only when the organism is
motile. Because of their greater refractibility, one may observe these
smaller forms in a fresh preparation under the low power lens. The
movement is much slower than in the larger form of ameba, and it
is possible to distinguish readily the extrusion of the pseudopodia, the
plasmic flow, and locomotion.
The nucleus of the small form of the non-pathogenic tetragenous ameba shows in general the characteristics of the small form of *Entamoeba tetragena* Viereck, but with a little experience a number of distinguishing characteristics are brought to light. The nucleus, as in other entamebae, is vesicular. Some specimens, owing to the coarse granules, appear irregularly formed. The nuclear membrane is thick, and the peripheral chromatin abundant, sometimes coarse and irregular, again occurring in a few large masses. In the center of the nucleus lies a relatively large centriole, which occasionally presents the picture of division. The cyclic phenomena described by Hartmann for *Entamoeba tetragena* Viereck do not take place. One frequently sees an organism with two nuclei, but never one with four.

*Cyst.*—The cyst is usually found in the formed or soft stools, and particularly after diarrhea. As a rule, this type of ameba forms cysts abundantly, while they occur but sparingly with the dysenteric ameba. When the non-pathogenic ameba is found in the feces, it is always possible to find numerous cysts also.

The cyst, which is highly refractile, is as a rule spherical, at times oval. In the process of transformation of the vegetative into the cystic form the volume of the cytoplasm is first reduced by an expulsion of foreign matter and fluids. It is thus rendered clear and transparent, and the meshy structure becomes distinct. The nucleus appears somewhat swollen and becomes rich in chromatin which accumulates in the center and along the outer periphery. A number of chromidia are seen in the cytoplasm. Simultaneously with these changes in the nucleus, appears a clear space, like a vacuole, in the cytoplasm (Fig. 11) which gradually increases in size (Fig. 12). In the stained preparations the nuclear content is seen to become gradually rich in chromatin, so that the large and small chromatin bodies surrounding the karyosome become visible. The chromatin along the nuclear membrane forms in masses, some of which proceed from the nucleus into the cytoplasm where they proliferate. These chromidia are located along the periphery or irregularly around central vacuoles (Fig. 13). One sees frequently that the chromidia and the nucleus are pressed against the nuclear wall because of the enormous dilatation of the central vacuole. In the course of time the elongated nucleus is constricted into two nuclei. In this process the central vacuole
decreases gradually in size and finally disappears, and the cytoplasm which previously had been crowded toward the periphery, now becomes voluminous again. The number of chromidia is also decreased. Some remain occasionally in the cytoplasm in the form of chromatin particles. In this stage it is easy to distinguish the cystic membrane.

The mature cyst contains four nuclei. Its diameter in fixed preparations averages from 6 to 12 μ, the size of the daughter nuclei being 1.2 to 2.5 μ. As Kuenen and Swellengrebel have described for the dysentery ameba, we can also trace in the cyst of the ameba under discussion a kind of supplementary nucleus, smaller than the normal nucleus. It has the appearance of spherical bits of chromatin. As a rule, the supplementary nucleus does not stain well, so that it can be distinguished readily from the normal nucleus.

The fact that the chromidia attain their maximal growth simultaneously with the appearance of the vacuoles, and that they disappear at the same time, points to the conclusion of their genetic relation.

Mitosis and Reproduction.—As Schaudinn has described for Entamoeba coli, the reproduction of the vegetative forms of the non-pathogenic tetragenous ameba also proceeds by simple division. We often see the large and small vegetative forms of the ameba harboring two nuclei (Figs. 3 and 9), which arise in the following manner. The original nucleus becomes enlarged to about twice its size. On the one side an increase in chromatin bodies takes place, while on the other the central karyosome divides into two parts which are united sometimes by a fine filament. The enlarged nucleus gradually becomes elongated, and is then constricted in the middle and divides by amitosis. The cytoplasm follows the same procedure. A typical spindle is rarely seen.

This simple cell division must be considered as the usual mode of reproduction of the non-pathogenic ameba. The forms having two nuclei are most numerous in diarrheal stools following purgation. From this we may conclude that this division plays a large part in the rapid proliferation of the ameba. Kuenen and Swellengrebel also observed this division which they have described minutely. Our findings are in accord with theirs.
In addition we have observed quaternary division, such as Schaudinn, Hartmann, and others have found in *Entamoeba coli* and described as schizogenesis. A nucleus is found to divide first into two, and then into four parts (Fig. 4). In the vegetative form we were unable to observe this process. After a certain length of time we have found four small amebae with four nuclei (Fig. 5) arising from the one organism. This process of division is found in amebae obtained from fluid stools, but as a rule less frequently than the division which produces two amebae. In experiments conducted previously with the dysentery ameba, we found frequently in the intestinal tissues of animals killed when the dysentery was at its height, amebae containing two nuclei, but rarely organisms harboring four nuclei.

The amebae produced by simple division or schizogenesis are on the whole very small, measuring only 6 to 11 μ. They have a relatively large nucleus which is plainly visible in fresh preparations. In the center is a nucleolus half or two-thirds as large as the nucleus; it stains intensely with hematoxylin. The nuclear membrane is thin and smooth, and not rich in chromatin. Between the nuclear membrane and the karyosome no chromatin is visible. The cytoplasm is of coarse, mesh-like structure. The endoplasm cannot be readily distinguished from the ectoplasm. No foreign substances are visible in the cell substance. The young amebae are very clear and transparent in fresh preparations.

The processes of encystment and cell changes are somewhat complex, but coincide in several points with those of *Entamoeba coli*. The ameba decreases in size, retaining, however, a meshy structure of the plasma, as in the vegetative form. It now contains no foreign material, so that at this stage the organism appears perfectly transparent. The refractile nucleus is easily recognizable. The nucleus then increases its chromatin, which accumulates in the center and along the periphery. In the cytoplasm one sees variously formed chromidia. Upon the expulsion of the chromidia, a vacuole appears in the middle of the cell substance (Fig. 11), which increases in the course of time and comprises finally the larger part of the cell substance. The cytoplasm and the chromidia are crowded into the small area along the periphery. This causes the nucleus to assume an oval shape; the centriole divides into two parts which are
gradually drawn toward the two poles. One often sees divided centrioles still attached by a fine thread. Typical spindles are often found. Upon the completion of nuclear division there is a decrease in the size of the central vacuole which finally disappears and the cytoplasm again becomes voluminous (Fig. 17). The chromidia also decrease in number, although they may remain for a long time as chromatin particles. In fresh preparations the cytoplasm is at this stage clear and transparent. The cystic membrane is distinct, appears double, and is markedly refractile. The two freed nuclei lie side by side, but isolated by chromidia. The mass of chromidia is larger than in other forms of amebae (Figs. 19 to 22). The two mature nuclei again divide amitotically, and we have as a result a typical cyst containing four nuclei. The four daughter cells within the cyst are isolated. Frequently one finds but few chromidia within the cyst (Figs. 39 to 42).

In addition to the manner of division described above, one may observe still another form. During the development of the cyst we see a somewhat swollen nucleus with a membrane having but little chromatin and having adherent to it a number of chromatin particles which stain intensely black with hematoxylin. This picture reminds one of Hartmann's degenerated form of dysentery ameba. After the disappearance of the nuclear membrane these chromatin particles lie together in the cytoplasm. In the end every chromatin particle is found to be a nucleus, measuring 1 to 1.5μ, smaller in size than that formed by division (Figs. 23 to 26). The centriole is often not visible. According to the above description we must assume a somewhat modified view respecting the origin of the daughter nuclei. The four isolated daughter nuclei originate from the mother nucleus after the disappearance of the nuclear membrane.

Another mode of division is frequently observed. The abundant chromatin bodies which have been developed in the nucleus wander out into the plasma, leaving but little chromatin in the mother nucleus which is finally resorbed in the plasma. The cast off, kidney-shaped chromidia then undergo change, and in due time form a nucleus. Surrounding the two chromidia is seen a narrow, clear zone (Figs. 27 to 30).
Remarks on Individuals Harboring the Non-Pathogenic Ameba.

After having had amebic dysentery the patient often evacuates cysts and has numerous relapses. It was conceivable that the organisms which we observed represented the cystic form of the typical dysentery ameba, and in fact we at first assumed this to be the case. We waited long for relapses of dysentery to appear, but notwithstanding the lengthy observation—8 months in one case—and the use of purgatives, our patients never developed symptoms of amebic dysentery. Repeated examinations of the rectal region by proctoscope failed to show pathological conditions. In only one instance did we observe slight catarrhal changes on the mucous membrane of the large intestine, but no inflammation or scars.

In our study of amebic dysentery we found that sixteen out of seventeen persons after convalescence become cyst carriers, and after the administration of purgatives often experience a recurrence of the disease. With the non-pathogenic tetragenous ameba the result is different. The patients failed to develop objective or subjective symptoms after repeated purgation.

Animal Experiments.

Viereck discovered in non-dysenteric stools amebae which develop tetranuclear cysts. Unfortunately he did not supplement his studies on this point by further publications. Russage believed the tetragenous ameba to be a form of the coli ameba, and denied its pathogenicity. Whether or not the tetranuclear cysts observed by Viereck and Russage are identical with the cyst of the tetragenous ameba described here, cannot be definitely affirmed, since animal experiments are not reported in the writings of Viereck and Russage. On the other hand, we have conducted such experiments, and the innocuous character of our ameba can, therefore, be demonstrated not only clinically but also experimentally with paralleled observations on the cysts of amebic dysentery.

Authors are divided on the question of the pathogenicity of the dysentery ameba. Kartulis, Juergens, Hartmann, Werner, and others claim to have proved its pathogenicity by experiments upon various animals, while a number of other investigators, such as Grassi, Cunningham, and Röhmer have denied this point. In a former study of experimental amebic dysentery in young cats, we introduced the dysentery ameba per anum and the cysts per os. Of 53 animals receiving the organism per anum 91 per cent proved positive; of 12 receiving them per os, 50 per cent were positive. The infected cats showed the typical symptoms of dysentery. The stools always contained bloody mucus and numerous amebae.
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Young cats of 400 to 600 gm. body weight were found to be most suitable for these experiments.

In a similar manner the author has conducted experiments with the new tetragenous ameba on twenty-three young cats of 400 to 600 gm. body weight. Of these, ten animals received the vegetative forms per anum, and thirteen animals the cysts per os. The feces used for inoculation came from three cases. In two of the animals receiving the amebae per anum, 4 days after injection we found the vegetative forms in the soft stools; these findings continued for about 4 days. Then, however, the vegetative forms disappeared gradually from the stools, without being replaced by the cysts. During the whole course of the experiment the animals showed no symptoms of dysentery, but remained well. After 10 days they were killed and autopsied. Nowhere in the intestinal mucosa did we find a dysenteric focus, and there were no amebae present in the tissues. Three of the other eight experimental animals received later several injections of the vegetative forms per anum. No evidence of infection could be found. The animals continued in a healthy condition.

In five of the thirteen animals fed with cysts, a few days later a small number of cysts was found in the feces. Only one of the cats evacuated after 6 days sluggishly motile vegetative forms, which, however, disappeared a few days later.

The amebae discharged by the cats are in general morphologically identical with those obtained from human feces. They are from 12 to 14μ in size. When at rest, it is difficult to distinguish between endoplasm and ectoplasm. The cytoplasm is coarse meshed and contains little foreign material. The nucleus measures usually 1.5μ in diameter. In the center is a relatively large centriole rich in chromatin. The movements and the plasmic flow of the organisms are very slow.

As a method of enrichment for the amebic cysts, we employed the antiformin or antiformin-ether method, as well as the Ujihara method. We often found several cysts that were injured. For that reason we used, as in the infection experiments with the cysts of the dysenteric ameba, a 0.6 per cent saline solution as an enriching medium. The fecal mass containing the cysts was dissolved in the salt solution,
and filtered through double gauze. The filtrate was centrifugalized and the sediment washed several times with the salt solution, until it became relatively clean and free from fecal odor. A large number of uninjured cysts may be obtained by this procedure. In earlier experiments we had found that the cysts washed with tap water also proved good infective material for cats.

**Morphological Differentiation of the Non-Pathogenic Ameba.**

*Entamoeba tetragena* Viereck.—Viereck, Hartmann, Werner, Akashi, Whitmore, Darling, and Kuenen and Swellengrebel have described this ameba in detail. The size of the large, vegetative forms is from 20 to 40 μ, the largest being 60 μ, while the smallest vegetative forms measure usually 20 to 25 μ. The specimens of our ameba are on the whole smaller than the dysentery ameba, the largest averaging from 17 to 25 μ, and the smaller forms from 6 to 19 μ. In contrast to the dysentery ameba, the motility of our organism appears sluggish, and the ectoplasm can be distinguished from the endoplasm only during movement. The plasmic flow is very slow and not smooth. Red blood corpuscles are never found in the cytoplasm. The nuclei of the dysentery ameba and the non-pathogenic tetragenous ameba are much alike, but with sufficient experience a number of characteristic differences can be detected, as have already been described. We desire here merely to emphasize that in differentiation one must have in mind the entire structure of the organism.

It is difficult to distinguish the cysts of the two types of amebae, and for this reason it is better to base judgment upon the vegetative forms than upon the cysts. The cysts of the dysentery ameba measure on the average 11 to 14 μ in diameter, those of the non-pathogenic tetragenous ameba 8 to 12 μ. Craig states that the cyst of *histolytica* has numerous chromidia, while the *coli* cyst harbors none; he differentiates the types of ameba on this basis. We do not agree entirely with this view, but it is certain that the chromidia in the non-pathogenic tetragenous ameba are smaller and fewer in number.

*Entamoeba coli* Lösch emend. Schaudinn.—The vegetative forms of the *coli* and the non-pathogenic amebae have the following points of resemblance. (1) Both are non-pathogenic for man and animals,
and may become parasitic in the normal human intestine. (2) The morphological characteristics, particularly the type of movement, the differentiation of the ectoplasm, and the structure of the nucleus, are alike up to a certain point. The two types of ameba can be readily distinguished, however, in their cysts. The cyst of *Entamoeba coli* measures from 14 to 19 μ in diameter; according to Kuenen and Swellengrebel, it measures 16 to 25 μ. Hence this cyst is much larger than that of the non-pathogenic organism. Mature *coli* cysts contain always eight daughter nuclei. The non-pathogenic ameba forms a cyst with four daughter nuclei, never eight.

Of other amebae—such as *Entamoeba minuta*, *hartmanni*, *williamsii*, *butschlii*, and *pilleti,*—*Entamoeba hartmanni* possesses the greatest similarity to our non-pathogenic ameba, though several points of difference are manifest.

*Entamoeba minuta* was so called by Elmassian on account of its small size. According to his description, it does not coincide with our ameba. Hartmann believed this type to be the same as *Entamoeba coli*. Kuenen and Swellengrebel, on the other hand, believe it to be identical with *Entamoeba tetragena*, with which viewpoint the author concurs.

*Entamoeba hartmanni*, n. sp., is distinguished from other *coli* amebae by its minute size (4 to 13 μ), the structure of the nucleus, and the markedly characteristic, thin, bacteria-like chromidia. This finding of von Prowazek coincides in some points with our own, without, however, being identical.

**CONCLUSIONS.**

The non-pathogenic tetrigenous ameba described here multiplies in the intestine of man, without producing any noticeable symptoms of dysentery.

This non-pathogenic ameba is distinguished morphologically and biologically from *Entamoeba tetragena* Viereck and *Entamoeba coli* Lösch emend. Schaudinn.

The vegetative forms of the non-pathogenic tetrigenous ameba may be divided into two groups, large and small. The large vegetative form is found in the normally evacuated or the diarrheal stools
obtained after purgation. The small form is found in the soft stools. This form may be transformed into cysts, or may reproduce itself by binary fission. Cysts are found in relatively large numbers in the soft and formed stools.

Experimentally, the new tetragenous ameba may be readily distinguished from the pathogenic variety. The vegetative forms of the dysentery ameba produced the disease in 91 per cent of cases when introduced per anum, and in 50 per cent where the cysts were introduced per os. Similar infection experiments undertaken on twenty-three young cats, i.e. ten experiments with the vegetative form of the non-pathogenic tetragenous ameba per anum, and thirteen experiments with the cysts per os, failed to produce pathological conditions. The intestines of the animals macroscopically and histologically failed to show any of the changes of dysentery.

Hence we may conclude that the new type of tetragenous ameba described here is non-pathogenic for man and for young cats.

We desire to express our appreciation to Professor R. Inada and Professor S. Ogawa for the assistance which they have afforded us in our work.

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**EXPLANATION OF PLATES.**

**PLATE 44.**

Figs. 1 to 4. Large vegetative form.

Fig. 1. Mononuclear ameba showing ingested masses.

Fig. 2. Same as Fig. 1, showing bacteria in vacuole.

Fig. 3. Binuclear ameba (simple division).

Fig. 4. Tetranuclear ameba (multiple division).

Fig. 5. Young amebae after schizogony.

Fig. 6. Nucleus and cytoplasm of large vegetative ameba.
Fig. 7 to 10. Small vegetative forms.

Fig. 7. Mononuclear ameba with distinct ectoplasm.

Fig. 8. Same as Fig. 7, showing bacteria.

Fig. 9. Binuclear ameba; nucleus with chromatin particles.

Fig. 10. Several chromidia in cytoplasm.

Figs. 11 to 18. Cyst formation.

Fig. 11. Mononuclear cyst with small central vacuole, showing cyst wall.

Fig. 12. Same as Fig. 11, with large central vacuole, in process of formation. Cyst wall fully developed.

Fig. 13. Same as Fig. 11. Nuclear division. Chromidia surrounding vacuole.

Fig. 14. Nuclear division without large central vacuole.

Fig. 15. Constriction of nucleus.

Fig. 16. Spindle formation of nucleus.

Fig. 17. Binuclear cyst; nuclei still close together. The central vacuole has disappeared.

Fig. 18. Same as Fig. 17, showing division of a nucleus.

Plate 45.

Figs. 19 to 42. Cyst formation.

Figs. 19 to 22. Simple division of nucleus.

Figs. 19 and 20. Simple division into two.

Figs. 21 and 22. Cyst with two nuclei, which have arisen after simple division.

Figs. 23 to 26. Nuclear division of degeneration forms.

Figs. 23 and 24. Nucleus with four chromatin particles at nuclear membrane.

Fig. 25. Swollen nucleus with four daughter nuclei.

Fig. 26. After the disappearance of the nuclear membrane, four daughter nuclei appear in the cyst.

Figs. 27 to 30. Four daughter nuclei arising from chromidia in cytoplasm.

Figs. 27 and 28. Enlarged mother nucleus and chromidia in cytoplasm.

Figs. 29 and 30. Four daughter nuclei formed from chromidia.

Fig. 31. Binuclear cyst. The nuclear membrane is thin; the nucleus contains several chromatin masses.

Fig. 32. Same as Fig. 31. Mass of chromidia.

Fig. 33. Trinuclear cyst, spindle formation, and division by reduction.

Fig. 34. Same as Fig. 33. Division of karyosome.

Fig. 35. Binuclear division. Double reduction.

Fig. 36. Nuclear division. Reduction.

Fig. 37. Spindle formation of two nuclei.

Fig. 38. Trinuclear cyst; one nucleus is very small.

Figs. 39 to 42. Tetranuclear cyst with and without chromidia.
(Shimura: Non-pathogenic tetragenous ameba. I.)
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