OBSERVATIONS ON CHROMATOID BODIES IN THE CYSTS OF ENTAMOEBA HISTOLYTICA

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OBSERVATIONS ON CHROMATOID BODIES IN THE CYSTS OF ENTAMOEBA HISTOLYTICA[1]

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[From the Gorgas Memorial Laboratory, Panama, R. de P.]

About a year ago, one of us (J. F. B.) noted that when cysts of *E. histolytica* were kept in water there would be seen a larger proportion with chromatoid bodies than when the cysts were allowed to remain in the fecal mass. While attempting to find the cause of this peculiarity it was discovered that chromatoid matter apparently occurs in two forms: As microscopically demonstrable chromatoid bodies, and as a microscopically invisible substance which has been termed latent chromatoid matter.

It is well known that *E. histolytica* cysts in evacuated fecal material gradually lose their chromatoid bodies. As early as 1912, Hartmann (1) noted that they were consumed during the development of the cyst and its subsequent life. Dobell (2) observed that the mature living cysts lost their chromatoid bodies in the course of a few days, and assumed that they were being absorbed. It will be shown that the loss of these primary chromatoid bodies is not entirely a process of consumption or absorption, but mostly a change from a manifest to a latent form of chromatoid matter from which a formation of secondary chromatoid bodies can be produced by transferring the cysts from the feces into water, saline solution, or, probably, any other aqueous solution which would not be destructive to the cysts.

MATERIAL AND METHODS OF EXAMINATION

In order to convey the true significance of our results the essential data on the material used and the methods of examination employed will be given. Twenty cyst-containing fecal specimens from 10 different cases of *E. histolytica* infection were studied. These cases and the specimens were selected. To guard against mistakes in identification of cysts in wet unstained smears no case who had concomitant infections with *E. coli*, *I. butschlii*, or *G. lamblia* was used. Large races of *E. histolytica* with cysts measuring from 12 to 14 microns were selected in order to facilitate the observations on the nuclear development, the number, size, and shape of the chromatoid bodies, and the changes of degeneration, all of which are not

[1] Read before the Medical Association of the Isthmian Canal Zone at its three hundred and fifty-fourth monthly meeting May 19, 1936.

so easily seen in the smaller races. In order to make it possible to find the necessary cysts without prolonged search only stools showing 25 or more cysts under a ⅞-inch coverglass were used. The fecal specimens were stored in cardboard ice cream containers with moistened blotting paper inserted in the lids to prevent drying. The various liquid mixtures were made in the proportion of about 10 gms of feces to 100 cc of the liquid.

The stools were first examined within 5 minutes to 1 hour after evacuation and then at intervals as required by the experiment until most of the cysts or all had degenerated or disappeared. In 8 series the cysts were examined only in wet unstained smears while in the other 12 series wet-fixed stained smears (iron-hematoxylin method) were also used. One hundred cysts or more were observed at each examination except toward the end of the experiments, when sometimes only a few cysts remained. When both wet and stained smears were used an equal number of cysts, usually 50, were studied in each preparation. In all, 34,280 cysts were observed in wet unstained smears and the presence or absence of chromatoid bodies, their number per cyst, and their characteristics noted. Records were also kept of the morphological changes of degeneration in the cysts as they grew old under the various conditions of the experiments. In the stained smears, 15,770 cysts were studied and a corresponding record made, as well as notes on nuclear development and glycogen vacuoles.

The relative value of wet and stained smears for a study of this nature may be mentioned in this connection. The presence of chromatoid bodies and their size and shape can be best studied in stained smears. In wet smears the chromatoid bodies in not a few cysts with glycogen or large vacuoles will not be seen even though the cysts may be examined most carefully, and chromatoid bodies in the shape of small granules, spicules, or filaments may escape detection even in cysts with the clear cytoplasm usually present after absorption of the glycogen. Our records show that stained smears of young cysts with abundance of glycogen will reveal up to 20 percent more cysts with chromatoid bodies than wet smears; under all conditions they reveal more chromatoid bodies per cyst. In old specimens with many cysts in advanced degeneration and disintegration the wet smears are more accurate for the determination of percentage of cysts with chromatoid bodies, since in the stained smears the degenerate cysts stain poorly or not at all and readily escape notice.

MANIFEST AND LATENT CHROMATOID MATTER

In order to illustrate the transition of chromatoid bodies into latent chromatoid matter and the development of secondary chromatoid

bodies, graphs have been made of three representative series of observations on these changes. *Series 12* (fig. 1). This series is presented to show the changes in chromatoid matter from one of the

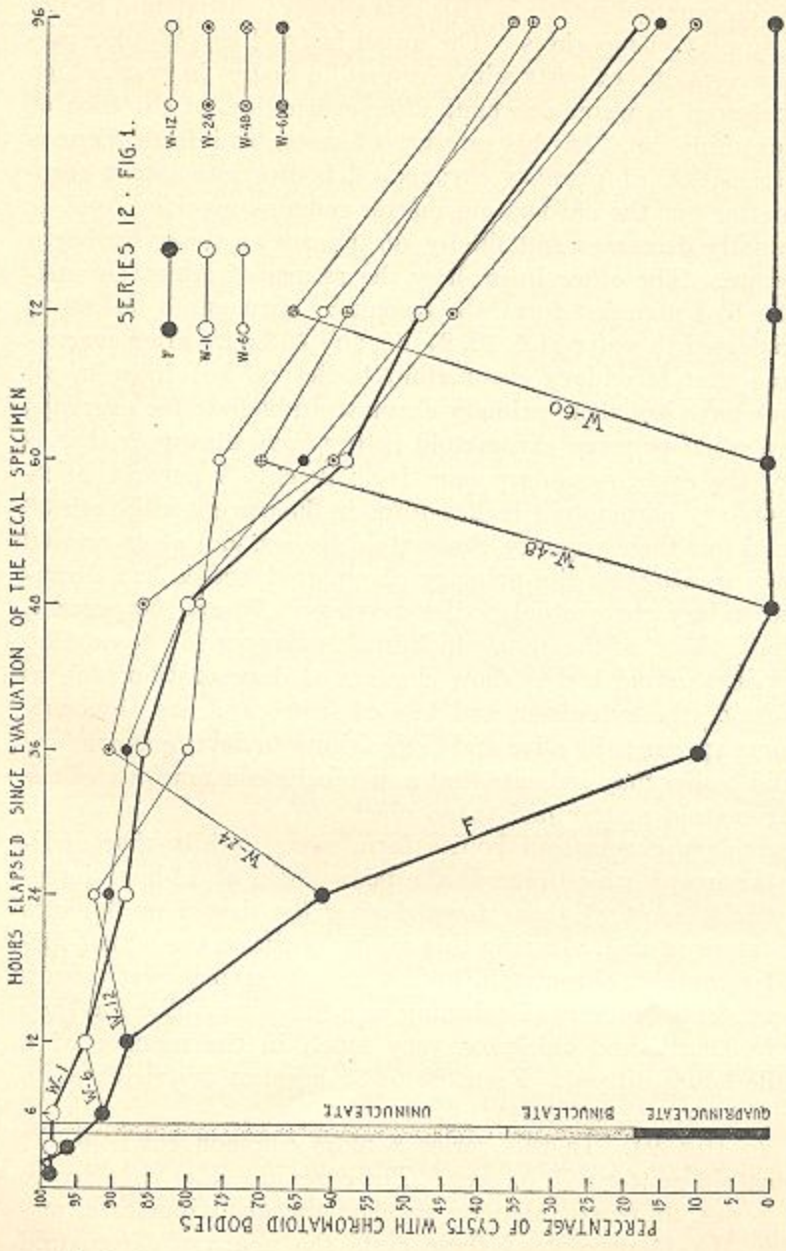

FIGURE 1.—A graph to illustrate changes in chromatoid matter in young cysts in which there has been practically no transition of primary chromatoid bodies into latent chromatoid matter before evacuation. Line F., transition of primary chromatoid bodies into latent chromatoid matter in the fecal material. Lines W–1, W–6, W–12, W–24, W–48, and W–60, cysts transferred from the feces to water at 1, 6, 12, 24, 48, and 60 hours, respectively, after evacuation of the feces; the increase in percentages of cysts with chromatoid bodies represents the transition of latent chromatoid matter into secondary chromatoid bodies. All specimens stored at room temperature.

earliest stages encountered in cysts from fecal material. The cysts were very young and mostly immature, 82 percent being uninucleate and binucleate. Practically all contained chromatoid bodies; in the first 3 smears, which were made 1, 2, and 3 hours after the evacuation,

only 2 of the 300 cysts observed were without chromatoid bodies. As shown in the lower heavy line (F) there is then a rapid decrease until at 48 hours all these primary chromatoid bodies have changed into latent chromatoid matter. This is the loss of chromatoid bodies observed by previous workers. The upper heavy line (W–1) represents the percentages of cysts with chromatoid bodies in cysts which were transferred to water one hour after evacuation, at the time of the first examination. In this mixture of water and feces there is no such transition of primary chromatoid bodies into latent chromatoid matter but the chromatoid matter remains manifest, and as such gradually decreases and finally disappears as the cysts begin to degenerate. The other lines show the return of latent chromatoid matter to a manifest form, the secondary chromatoid bodies, in cysts transferred to water at 6, 12, 24, 48, and 60 hours after evacuation. Note that secondary chromatoid bodies do not form in all cysts which have lost their primary chromatoid bodies; for example, at 6 hours when primary chromatoid bodies have disappeared in 8 percent of the cysts, secondary ones form in only 2 percent, at 24 hours secondary chromatoid bodies form in 30 percent although 36 percent had lost their primary chromatoid bodies, and at 48 and 60 hours when in all cysts the primary chromatoid bodies had disappeared, secondary chromatoid bodies develop in 70 and 66 percent, respectively. Most of the cysts which in this manner fail to develop secondary chromatoid bodies show changes of degeneration such as granulation of the cytoplasm and loss of lustre and are obviously dead; others appear to be alive and their failure to develop secondary chromatoid bodies may indicate that a metabolic consumption of the latent chromatoid matter had taken place.

The secondary chromatoid bodies form more rapidly than indicated by the graph which records the percentages at 12-hour intervals. Actually most of them form during the first 2 or 3 hours (at room temperature). During this rapid transition the actual formation of secondary chromatoid bodies may be seen in wet smears by the persistent observer. Beginning as a fine thread-like rod they increase in length and thickness very much in the manner of a wax candle being dipped. From 20 to 30 minutes are required to complete the formation (fig. 4).

Series 15 (fig. 2). In this series a more common condition of the chromatoid matter will be seen. The cysts are older than in the previous series, 97 percent having reached the quadrinucleate, mature stage. In 14 percent of these cysts the primary chromatoid bodies already had disappeared when the cysts were first examined, 10 minutes after evacuation (line F). As shown in W–⅙ almost all of these cysts had enough latent chromatoid matter to develop secondary chromatoid bodies. Transition of primary chromatoid bodies

into latent chromatoid matter apparently then had occurred while the cysts were retained in the colon. In most fecal specimens such pre-evacuation transition takes place to a greater or lesser extent.

The graph of this series has the usual pattern and presents nothing essentially new. Note, however, the quick transition of primary chromatoid bodies into latent chromatoid matter and the rapid loss of the latter and of the secondary chromatoid bodies. This could

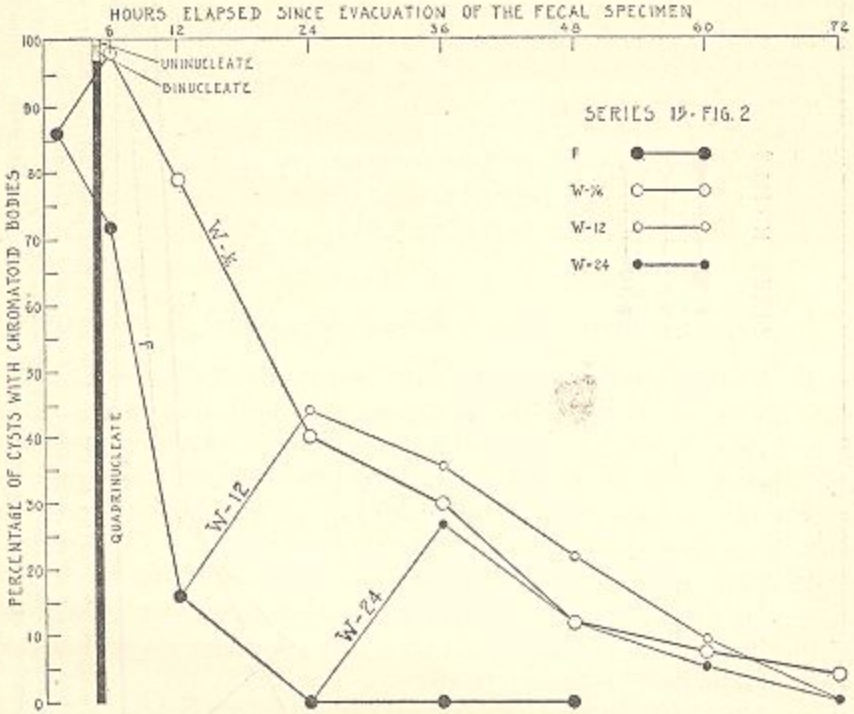

FIGURE 2.—A graph to illustrate the changes in chromatoid matter in older cysts in which some transition of primary chromatoid bodies into latent chromatoid matter has taken place before evacuation of the feces. Line F, transition of primary chromatoid bodies into latent chromatoid matter in cysts remaining in the feces. Lines W-½, W-12, and W-24, cysts transferred from feces to water at 10 minutes, 12 and 24 hours respectively after evacuation of the feces; the increase in percentage of cysts with chromatoid bodies represents the transition of latent chromatoid matter into secondary chromatoid bodies. All specimens stored at room temperature.

be expected to occur in these cysts which at the time of evacuation were relatively old. Degenerative changes appeared correspondingly early, for example, at 48 hours very few cysts remained in the feces and of these 70 percent were in a state of disintegration. It is not assumed, however, that the age of the cysts was the only factor in the early degeneration of the cysts in this stool. Equally mature cysts, and probably as old, have been seen to last much longer in other specimens of feces stored under the same conditions. Biological variation in various strains, differences in the putrefactive process of the feces, and probably other conditions are undoubtedly factors

influencing degeneration in the cysts and incidentally the changes in chromatoid matter.

Series 14 (fig. 3). This series is presented to show the exteme to which the chromatoid matter may disappear before evacuation of

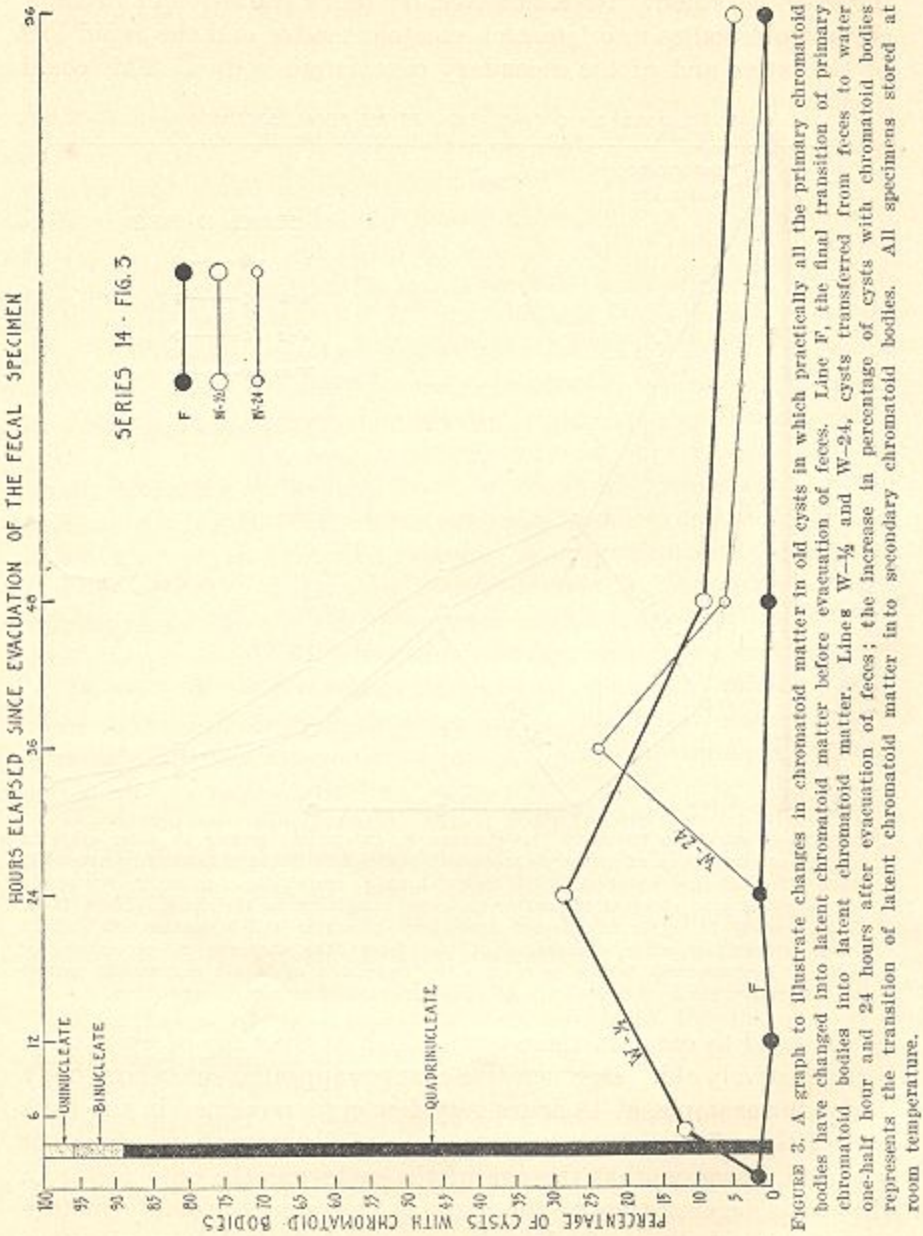

FIGURE 3. A graph to illustrate changes in chromatoid matter in old cysts in which practically all the primary chromatoid bodies have changed into latent chromatoid matter before evacuation of feces. Line F, the final transition of primary chromatoid bodies into latent chromatoid matter. Lines W–½ and W–24, cysts transferred from feces to water one-half hour and 24 hours after evacuation of feces; the increase in percentage of cysts with chromatoid bodies represents the transition of latent chromatoid matter into secondary chromatoid bodies. All specimens stored at room temperature.

the cysts. The cysts were obtained from a formed dry stool which obviously had been retained a long time in the colon. When first examined, one-half hour after deposition, chromatoid bodies were

found in only two cysts (2 percent), in one a short rod, and in the other a slender rod and a thin filament. Transferred to water at this time (W–½), only 28 percent of the cysts developed secondary chromatoid bodies; 24 hours later, 23 percent still had enough latent chromatoid matter to develop secondary chromatoid bodies (W–24). When the pattern of this graph is compared with series 15 it appears reasonable to assume that the chromatoid matter in the cysts of series 14 at the time of evacuation was in the same stage as the chromatoid matter in the cysts of series 15, 24 hours after deposition, at which time the primary chromatoid bodies had disappeared and latent chromatoid matter was present in 27 percent of the cysts. The character of the stools from which the cysts of the two series were obtained supports this assumption; the stool used in series 15 was formed but soft, while the stool for series 14 was formed and dry, and obviously a stool of constipation which well may have been retained 24 hours longer in the colon.

CHANGES IN CHROMATOID MATTER UNDER VARIOUS CONDITIONS

Temperature.—In the three series of observation discussed above all specimens were kept at room temperature, 28° C. to 32° C. The changes in chromatoid matter also have been studied in cysts stored in incubator at 37° C., and in refrigerator at 10° C. Except for variations in time the changes were essentially the same. At 37° C. the transition of primary chromatoid bodies into latent chromatoid matter required from 10 to 12 hours, at 28° C. to 32° C. from 24 to 48 hours, and at 10° C. from 11 to 12 days. Latent chromatoid matter could be demonstrated (by transfer of cysts to water and noting development of secondary chromatoid bodies) for 24 to 48 hours in cysts kept at 37° C., for 60 to 72 hours in cysts at 28° to 32° C., and for 17 to 18 days at 10° C. Secondary chromatoid bodies lasted a short time beyond these periods due, apparently, to the longer life of the cysts in water.

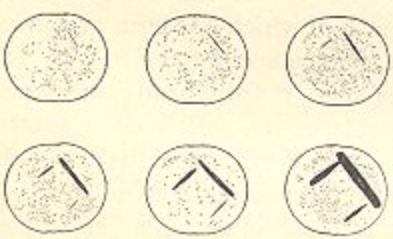

FIGURE 4.—The formation of secondary chromatoid bodies in an *E. histolytica* cyst observed in a wet smear with 4 mm. objective and 6X ocular .X1000. The sketches were made at intervals of 5 minutes.

The transition of latent chromatoid matter into secondary chromatoid bodies is strikingly accelerated at 37° C. and much delayed by refrigeration. The change is also more complete at higher temperatures, as indicated by the development of more and larger secondary chromatoid bodies.

Reaction.—During the early stages of this work the findings seemed to indicate that acidity and alkalinity influenced the changes in chromatoid matter. Comparative observations were then made on cysts in neutral, acid, and alkaline mixtures of feces and water. Hydrochloric acid, 1 percent, and sodium hydroxide, 1 percent, were used for adjusting the reaction which was roughly determined by litmus paper. It was found that slight deviations from the neutral point had no apparent influence on the changes in chromatoid matter. In strongly acid mixtures latent chromatoid matter developed into more abundant and often larger secondary chromatoid bodies than in neutral mixtures, while in corresponding strongly alkaline mixture the cytoplasm of the cysts became opalescent and lifeless, the nuclear chromatin coalesced, and secondary chromatoid bodies failed to develop, all apparently due to injury to the cyst wall allowing alkalinization of the cytoplasm and rapid degeneration.

SUMMARY OF THE CHANGES IN CHROMATOID MATTER IN *E. HISTOLYTICA* CYSTS

The results recorded above and some incidental observations make it possible to trace chromatoid matter from the time of its first appearance in the newly formed cysts until its disappearance in the disintegrating cysts. As already has been shown, changes in chromatoid matter take place before as well as after evacuation of the cysts, and since most of the stools containing cysts are formed, having been retained in the colon for a considerable time, these changes are usually well advanced even when the cysts are examined immediately after deposition. In order to see all events in one batch of cysts it is necessary to select a stool which has been evacuated with a minimum delay in the bowel. Since such stools will be soft or mushy they usually contain only, or mostly, trophozoites, but occasionally patients with subacute amoebic colitis will pass a shower of cysts in a mushy stool. These young cysts, particularly the uninucleates and binucleates, if examined immediately, will present chromatoid matter in the earliest phases observable in human infections.

The young cysts have an abundance of chromatoid matter, manifest in the most variable form and greatest numbers per cyst seen in any stage. Most cysts have four or more, and not a few have their cytoplasm crowded with chromatoid bodies. More than 30 have been counted in some. With four or less in a cyst they usually have the characteristic rod shape, but when more numerous they frequently are in the form of minute filaments, spicules, wedges, or granules. In cysts with large glycogen vacuoles, which will be found in the majority of these young cysts, the chromatoid bodies are usually small

and numerous and crowded against the periphery; with less glycogen they frequently are packed into thick nondescript masses.

When the cysts are allowed to remain in the fecal mass these primary chromatoid bodies gradually disappear as they change into latent chromatoid matter. The time required for this process varies with the temperature at which the stool is kept, as noted above. During this period of transition into latent chromatoid matter the chromatoid bodies invariably become fewer per cyst, the larger rod-shaped ones being the last to disappear. At any time during and subsequent to the disappearance of the primary chromatoid bodies and until the cysts begin to show morphological changes of degeneration, the latent chromatoid matter returns to a manifest form (secondary chromatoid bodies) when the cysts are transferred to water. This transition also takes place in saline solution, Ringer's solution, in liquids used for culturing *E. histolytica*, such as Locke albumen for the Boeck-Drobhlav medium, and the horse serum and saline for covering agar slants, and probably in any aqueous solution which is not injurious to the cysts.[2] The formation of secondary chromatoid bodies is accelerated when the cysts are kept at 37° C. and delayed at lower temperatures. When observed in wet smears at room temperature the transition of latent chromatoid matter into secondary chromatoid bodies, once started, has been seen to require only from 20 to 30 minutes. The secondary chromatoid bodies vary in number, size, and shape according to the amount of latent chromatoid matter present in the cysts at the time of their formation. With an abundance of the latter they become long thick rods or bars, brilliantly refractile in wet smears, while with a scantier supply they form as thin rods or filaments, or broad rods or bars of a very light density which in wet smears lack the bluish luster and appear glasslike. In general the secondary chromatoid bodies show less variation in shape and frequently are diagramatically typical of the characteristic chromatoid body of *E. histolytica*. Broad, thick bars extending along the whole diameter of the cyst are particularly distinctive.

As cysts grow old there is a steady loss of chromatoid matter whether latent as in cysts kept in feces or manifest as in the cysts transferred to liquids. When latent this loss becomes evident in the decreasing number and lessened density of the secondary chromatoid bodies developing upon transfer of the cysts to water; when manifest they can be seen to decrease in size and in number per cyst. The final disappearance of chromatoid matter is incident to morphological changes of degeneration observable in the unstained cyst (granulation of the cytoplasm with loss of lustre and coalescence of the pe-

[2] Yorke and Adams (9) may possibly have observed the transition of latent chromatoid matter into secondary chromatoid bodies when they noted an "initial increase" in percentage of cysts containing chromatoid bodies (from 27 to 56 percent in 3¼ hours) during cultivation on Locke-egg-serum medium.

ripheral nuclear chromatin) or it may precede these obvious changes of disintegration by a few hours.

COMMENT ON SOME OF THE RESULTS RECORDED BY PREVIOUS OBSERVERS

Origin of chromatoid bodies.—Hartmann (1) held that the chromatoid bodies have their origin in the nucleus. James (4), after a prolonged study of their staining reactions and their morphological characteristics, came to the conclusion "that they are derived from the cytoplasm by a process of condensation in the latter, and have nothing whatever to do with nuclear chromatin." Dobell (2) states that he had not found that staining reactions proved their cytoplasmic origin. Whether they are formed from the chromatin of the nucleus or are secreted in the cytoplasm, he considered uncertain, and all that he could say with certainty was that they appeared in the cytoplasm.

Having observed their actual formation we can state with certainty that in their earliest visible form they appear in the cytoplasm without any microscopical evidence of any relationship to nuclear chromatin and that the process of formation aptly can be described as "a process of condensation" of some substance in the cytoplasm.

Function of chromatoid bodies.—The function of chromatoid bodies never has been definitely determined. To Hartmann (1) it appeared that they acted as *Reservestoff* since they would be consumed during the development of the cysts and the following resting stage. Dobell (2) also considers it probable that, like the glycogen, they represent reserve material of some sort. He holds the same opinion in 1927 (3), then stating that "it seems reasonable to suppose that glycogen and chromatoids are reserve substances (carbohydrate and protein, respectively) which the amoebae store in their cysts to enable them to live outside the body, when they are no longer free and able to capture food." Not a few writers subsequently have expressed the same opinion.

Although the disappearance of the primary chromatoid bodies, which phenomenon has been the basis for the food reserve theory, apparently does not represent consumption but merely a transition to another form (latent chromatoid matter), there is, nevertheless, a gradual decrease in the total amount of chromatoid matter, whether latent or manifest, a decrease which may be due to consumption in metabolic processes of the living cysts rather than to destruction incident to degenerative changes. A change in chromatoid matter which does not make it appear as stored food is the transition of latent chromatoid matter into the solid form of secondary chromatoid bodies when the cysts are incubated (liquid culture media, or solid media with a superimposed liquid). One would rather expect

that the chromatoid matter would remain in the latent form and thus be more readily available for consumption in the enhanced metabolism of incubation.

Frequency of occurrence of chromatoid bodies.—As indicated by the results presented above, the frequency of occurrence of chromatoid bodies in cysts from feces, even when examined immediately after evacuation, may vary from 100 to 0 percent, the variation depending upon the time elapsed since the cysts were formed. In stools which have been evacuated with a minimum delay the cysts may have formed so recently that all may still contain some of their primary chromatoid bodies, while in hard dry stools, which may have been retained in the colon 12 to 24 hours or even more and thus having permitted the primary chromatoid bodies to change into latent chromatoid matter, all cysts may be without chromatoid bodies. Usually stools are passed before the majority of the cysts have lost their primary chromatoid bodies.

Smith (5) found chromatoid bodies present in 27 percent, absent in 65 percent, and doubtful in 8 percent of 1,162 cysts examined in iodine solution. He considers it possible that very small chromatoid bodies might have been overlooked and that only counts from stained preparations would give absolutely accurate results. It has been our experience that even chromatoid bodies of average size readily may escape detection not only in iodine solution but even in saline, "in which medium", as Smith states, "the chromatoid bodies are more distinctly seen than in iodine." Furthermore, Smith gives no information as to the consistency of the stools, nor as to the time after evacuation that they were examined. Without these data his figures have no significance. Dobell (2) describes the formation of chromatoid bodies as part of the normal development of the cysts of *E. histolytica*, but also notes that "sometimes they are completely absent in cysts at all stages of development." The last statement is not understood to mean that cysts form and develop without at any time having chromatoid bodies, but that sometimes chromatoid bodies are completely absent in cysts which at the time of examination were in all stages of nuclear development. This condition is seen occasionally in cysts immediately after evacuation (see series 14) and frequently 24 hours or more later. Uninucleate and binucleate cysts without chromatoid bodies, although immature in their nuclear development, are thus relatively old cysts with delayed nuclear development in which the primary chromatoid bodies already have changed into latent chromatoid matter.[3] This conclu-

[3] It is, of course, possible that immature cysts may be passed before chromatoid bodies have developed. That this may occur is suggested by an observation made by Cleveland and Sanders (6) who noted that in cysts produced in cultures chromatoids sometimes appeared much later than they did at other times. They have drawings of uninucleate and binucleate cysts in which chromatoids have not appeared.

sion is based on the observation that the various changes in chromatoid matter take place irrespective of nuclear development. An example of this can be found in series 12; ~~No. II,~~ When the cysts were first examined, 1 hour after evacuation, 64 percent were uninucleate, 18 percent binucleate, and 18 percent quadrinucleate and all had chromatoid bodies; 48 hours later chromatoid bodies were completely absent in all cysts although 64 percent still were uninucleate and binucleate, and when transferred to water secondary chromatoid bodies appeared in cysts at all stages of nuclear development. Kofoid et al. (7) state that chromatoid bodies are found in a majority of the cysts, and Craig (8) finds that they occur in at least 50 percent of all cysts of *E. histolytica*. Rightly, Craig also has noted that "these bodies are most numerous in the uninucleate and binucleate cysts." A very different observation is recorded by Yorke and Adams (9): "Careful examination of freshly passed faeces", they state, "showed that these bodies were comparatively rarely present in the uninucleate cysts, but were commonly found in the bi- and quadri-nucleates", and again in discussing the variations in cysts passed at different times: " * * * on one occasion the cysts may be practically all uninucleate with much glycogen, and but few of them containing chromatoid bodies, whereas on another occasion the vast majority may be quadrinucleate with chromatoid bodies but little glycogen." These are indeed the findings when cysts are examined in iodine, which method apparently these workers used. Even the most careful examination of cysts stained with iodine will not reveal the small chromatoid bodies frequently present in uninucleate cysts with glycogen vacuoles. Often they are not seen even in unstained cysts in wet smears. Actually uninucleate cysts have as much chromatoid matter as binucleates and quadrinucleates of about the same age.

PRACTICAL APPLICATION

The change of latent chromatoid matter into secondary chromatoid bodies has a practical application in the laboratory diagnosis of amoebiasis. Not infrequently one encounters specimens of feces with cysts which should be suspected of being a small race *E. histolytica* but which cannot be identified in wet smears. For example, there may be numerous cysts of *E. nana* or of *C. mesnili*, and among these a few rounded cysts, perhaps a little larger and perhaps slightly different in their cytoplasmic structure, but not labeled with a chromatoid body. Smears in iodine solutions may not be of much help since the nuclei in the cysts of a small race of *E. histolytica* have such fine peripheral chromatin rings that their appearance in iodine often does not differentiate them from *E. nana*. Similarly a few small

cysts of *E. histolytica* in a shower of cysts of *I. bütschlii* may very readily escape detection unless they happen to have a characteristic chromatoid body. In wet-fixed stained preparations the differentiation can be made, of course, but when the cysts are very few, an unduly long search may be required. Not infrequently the simpler procedure will be to macerate a portion of the feces, about the size of a bean in a test tube of water and after 4 hours or later examine the sediment, or a concentrate thereof, and look for cysts with chromatoid bodies. Should concentrations be used, a method which does not require any chemical which is destructive to chromatoid matter must be selected. In this connection it may be mentioned that the sugar solution used in the concentration method by Yorke and Adams (9) does not affect the chromatoid bodies in any visible degree.

SUMMARY

Chromatoid matter in *E. histolytica* cysts apparently occurs in two forms, manifest and latent. This assumption is based on the observation that in cysts which have lost their chromatoid bodies while in the fecal material, a new set of chromatoid bodies may develop when the cysts are transferred from the feces into water. The hypothetical substance into which the primary chromatoid bodies are converted and out of which the secondary chromatoid bodies are formed has been termed latent chromatoid matter.

The transition of primary chromatoid bodies into latent chromatoid matter takes place in the cysts as they grow old in the fecal material, before as well as after its evacuation and apparently without any relation to nuclear development.

The formation of secondary chromatoid bodies has been observed and appears to be a process of condensation of some substance in the cytoplasm. They have essentially the same appearance as primary chromatoid bodies but tend to be more characteristically rod shaped and bar shaped.

Chromatoid matter, whether manifest or latent, decreases as the cysts grow old and disappears when they begin to show morphological changes of degeneration, or shortly before this stage.

The knowledge of these changes makes it possible to correlate or reasonably explain some of the data on the occurrence of chromatoid bodies recorded by previous workers.

The generally accepted theory that chromatoid bodies constitute a food reserve appears rather doubtful in view of some observations on these changes. Further studies on the transition of manifest chromatoid matter into latent and its return to the manifest form may reveal its significance in the metabolism of the cysts.

The change of latent chromatoid matter into secondary chromatoid bodies has a practical application of considerable importance in the laboratory diagnosis of amoebiasis.

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