STUDIES IN MICROBIC HEREDITY

V. THE BIOGENETIC LAW OF HAECKEL AND THE ORIGIN OF HETEROGENEITY WITHIN PURE LINES OF BACTERIA[1]

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Many years ago the biologists, Haeckel and von Baer, made a most interesting generalization which stated that the life history of the individual of a species recapitulated the life history of the race—or in another form, that ontogeny recapitulates phylogeny. This law is still regarded by biologists as valid in a large way, although it cannot be successfully applied in detail, for the simple reason that entire phyletic stages may be suppressed or new non-phyletic adaptive stages interpolated, in a manner quite misleading to one who would unravel the phyletic or racial history of a species from its individual ontogeny.

The general method proposed by these investigators has been particularly fruitful, however, in our studies on the genetic relationships of bacteria. This method consisted, in brief, of making observations over a course of years on selected species (?) isolated from a single cell. For this purpose cultures to be suitable must exhibit a life history expanded into several clean-cut morphologic phases capable of ready reproduction. In other words there must be a clear delineation of the pleomorphic cycle. Figure 1 and diagram 1 show that our K A. diphtheroid strain answered fully this qualification. (See bottom of page 204 for explanation of plate 1.)

Figure 1 at *a*, for example, shows the "normal" or better the dominant phase, in the ontogeny of this culture. This is a rather slender, non-granular, Gram-negative bacillus. At *b* we

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have a very coarse fungoid branching phase which gives rise to the gonidial and zygospore phases, *c* and *C*, and which is Gram-amphophilic. At *d* is a rudimentary branching phase not so coarse as *b*, and at *e* (diagram 1) is another bacillary phase which differs from *a* in being more retentive for the Gram stain. These represent the principal phases in the ontogeny of a pure line culture of this strain. To many bacteriologists it is still known only as the pleomorphic cycle and as such is without general biologic or medical significance.

Haeckel's Law suggests the possibility that these ontogenetic phases may stabilize under certain conditions as stages, variants or races—if one pleases—of this single organism. So stabilized they would then comprise its phylogeny. Indeed this result has been realized experimentally through the mechanism of the reorganization occurring in the sexual cycle. The first results with this strain were published several years ago (Mellon, 1920). The further experimental development of its phylogenetic history is the chief purpose of the present paper.

Previous work with the K. A. strain indicated clearly that under the conditions of the experiment it was possible to develop

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PLATE I

ONTOGENY

Fig. 1. K. A. 95.

DIAGRAM 1. The small circle at the left hand side of the diagram indicates that this strain may be monophasic, i.e., it may multiply apparently to exclusion in the bacillary or so-called normal phase—*a* (Figure 4). The progressive widening of the circles to the right to include phases *e*, *d*, *b* and *c*, indicates that the culture may be bi-, tri- or tetraphasic, etc., and that each of these is capable of reverting to the original phase *a*.

PHYLOGENY

FIG. 3. K. A. 257 variant. Stabilized phase *d* of figure 1.
FIG. 4. K. A. 191 variant. Stabilized phase *e* of diagram 1.
FIG. 5. K. A. 105 variant. Stabilized phase *C* of figure 1.
FIG. 6. Fungoid phase *b*. Not stabilized.

Table of magnifications for photographs

× 1200: Figures 1, 2, 4, 6, 7, 10 and 11
× 1500: Figures 5, 20 and 21
× 2500: Figures 8, 9 and 19
× 3000: Figures 12, 13, 14, 15, 16 and 17

FIG. 1

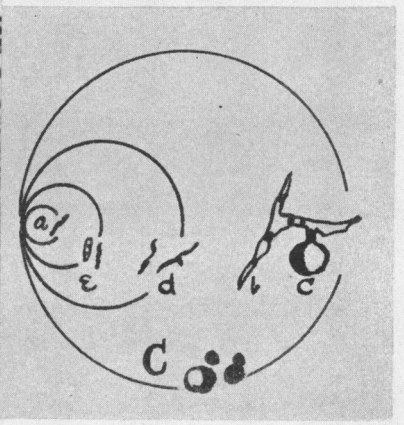

DIAGRAM 1

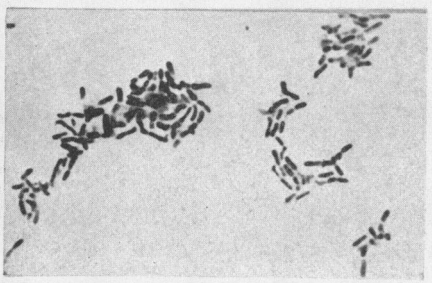

FIG. 3

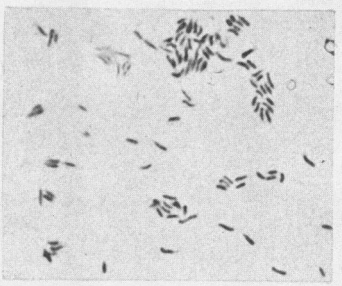

FIG. 4

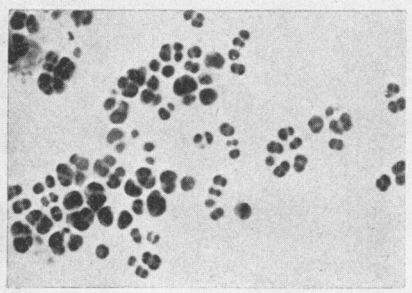

FIG. 5

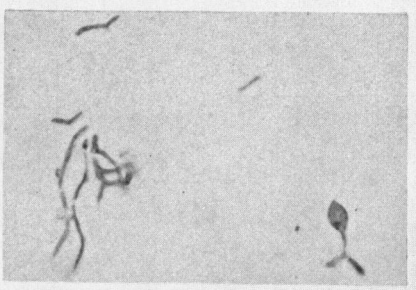

FIG. 6

(Mellon: Studies in microbic heredity)

large numbers of what we spoke of as "giant coccoids," and which we now regard as probable zygospores (1920). The original culture of this pleomorphic strain (fig. 1) will be known as K. A. 95. It was further shown to be possible for these large forms to undergo a double segmentation and yield a true coccus mutant, which will be known in this study as K. A. 105 (fig. 5, plate I). It is now desired to show that the same or similar forms (fig. 2, plate II) will, under other conditions, give rise to a bacillary type of mutant (K. A. 257) (fig. 3, plate I); although the latter is closely related serologically to the antecedent bacillus, their cultural and morphologic characters are so different that one is surprised that this is the case.

EXPERIMENT I: THE MECHANISM OF MUTATION YIELDING STRAIN K. A. 257

The strain in question was isolated in single cell culture by Dr. M. A. Barber in 1919 and was planted in ordinary glycerol broth 0.5 per cent, having a pH of 6.8. As grown at 37° it produced many very large, rather poorly staining giant coccoid forms, originating from the coarse branching phase of the culture (fig. I, *b* and *c*). It was placed in the ice chest for about nine months which caused the broth to evaporate more than one-half. Owing to the lack of viability of other transplants of this strain, this culture was resorted to in an effort to rejuvenate it. At this time it had always grown on Loeffler's at 37° but transplantation under these conditions failed. Placed at room temperature for a week a luxuriant growth of a new type of bacillus was obtained on the Loeffler's slant. Microscopic examination of the old broth cultures showed that apparently no bacilli remained and that it consisted entirely of very deeply staining coccoid forms which varied greatly in size (fig. 2, plate II). The lack of the usual intracellular staining differentiations in these forms may possibly be correlated with the new type of bacillus evolved from them—a very different race from the coccus mutant referred to above.

The large numbers of colonies which germinated on the slant at room temperature left little doubt that the resulting bacillus germinated from these coccoid forms, particularly since none of

the original bacilli appeared. The origin of a new race from these coccoids was definitely proven for another strain of the diphtheria group (1922). The K. A. bacillus, as we originally obtained it, grew sparsely and was Gram-negative (phase *a*), while the large coccoid forms (phase *C*—zygospores) retained the Gram stain decidedly longer and could almost be called Gram-positive, while the variant bacillus which grew at room temperature was also very definitely Gram-positive as was the coccus mutant mentioned above.

Culturally the mutant bacillus, K. A. 257 (fig. 3) grew much more luxuriantly than the original strain, K. A. 95, and developed a buff colored pigment, while the original strain some years later

TABLE 1

	K. A. 257	K. A. 105	K. A. 95	K. A. 191
	pH	*pH*	*pH*	*pH*
Glucose	4.30	5.4	5.3	5.3
Mannitol	7.4	5.7	7.2	7.1
Levulose	4.29	5.6	5.2	5.3
Salicin	5.67	5.6	6.8	6.8
Dextrin	7.4	5.6	6.9	6.8
Inulin	7.4	6.6	7.0	7.0
Sucrose	4.44	5.6	5.7	5.6
Maltose	4.47	5.7	5.7	5.9
Lactose	7.6	6.4	6.9	6.9
Glycerol	7.6	6.8	7.0	7.0

developed a yellowish pigment. Furthermore, the variant strain was very different morphologically in that it showed a marked tendency to rudimentary branching forms, was quite granular, and culturally had a rather wrinkled, more cohesive character than the original bacillus, which was moist and shiny. These cultural differences were furthermore associated with the acquisition of the fermentation of salicin, as is seen in table 1, which gives the sugar reactions of the various strains.

EXPERIMENT II: THE ORIGIN OF STRAIN K. A. 191

Strain K. A. 191 (fig. 4) was derived from the pleomorphic K. A. 95 as follows: The pure line K. A. 95 had been kept on a blood

agar slant for some months in the ice chest. The growth was luxuriant but the pleomorphism slight, as was the rule on this medium. The rather fitful viability of this strain has already been mentioned, and it was notoriously weak on blood agar in contrast to a prolonged viability on Loeffler's medium where the gonidial or giant coccoid forms were usually numerous.

Most of the attempts to revive the blood agar strains failed whether transfers were made at 37° or 20° and regardless of the media tried; but for some unknown reason one tube which had failed to grow at 37° did finally grow at 20° on a blood agar slant. Morphologically the strain was identical, and it was at first thought that the original strain had been recovered; but the fact that it gradually developed a yellow pigment, even in young cultures, that it also grew luxuriantly on Loeffler's medium where its pleomorphism was greatly diminished, that it failed to develop numerous gonidia (giant coccoids) in serum broth, and that it was definitely—if not strongly—Gram-positive, made it clear that we were dealing with a variant.

Serologically it proved to be mutually reciprocal with K. A. 95, but the fact that it failed to form the large gonidia (zygospores) in appreciable numbers made it unsuitable for the coccus mutation experiments as originally described for it. Here is a fact of the *greatest importance in mutation experiments* because it may well account for the failure of one observer to repeat the work of another when he *fancies* he is working with the identical strain, merely because it is serologically identical.

When it is better appreciated that mutation is not apt to take place except through the medium of a special developmental stage of the culture, attention will be directed to developing an environment that will evoke such a stage. If this cannot be done, the culture is apt to remain very stable. Strain K. A. 191 is one of the few variants in which I could not be sure of the mechanism because the pleomorphic forms were not present in sufficient numbers to trace it readily.

Phylogenetically it is apparent that K. A. 191 (fig. 4) represents a stabilization of stage *e*) of the ontogenetic cycle (fig. 1 and diagram 1); K. A. 257 (fig. 3) represents stage *d*; K. A. 105 (fig.

5) represents stage *C*. The coarse fungoid stage *b* (fig. 6) has never been stabilized with this species, although as reported a number of years ago a true streptothrix stage of a fuso-spirillary complex was stabilized (Mellon, 1919a).

EXPERIMENT III: THE EFFECT OF SELECTIVE ENVIRONMENT IN THE DEVELOPMENT OF THE PLEOMORPHIC PHASES OF THE K. A. STRAIN

Of a wholly different order from this *permanent* stabilization of morphologic type, is the *temporary* reproduction that can be induced in these ontogenetic (pleomorphic) phases by a selective environment. Yet the latter is of importance if for no other reason than as conclusive proof that these pleomorphic forms are capable of multiplication. In such event they cannot properly be viewed as involution forms.

For example the rudimentary branching stage *d* in the original culture will reproduce *as such* to a limited extent if the pleomorphic mixture is transplanted to tall tubes of glucose ascitic broth where the conditions are largely anaerobic. Stage *a*, which is the normal bacillary stage, will not reproduce noticeably under these conditions. Transfer of these forms to the original conditions brings a return of the original picture. *No stabilization has occurred.*

We have frequently observed, too, the temporary reproduction of phases *b* and *C* under the following conditions. These phases developed readily on Loeffler's slants at 37° and seemed directly connected with the prolonged viability of the organisms, since old cultures on media which did not yield phase *C* were usually non-viable.

Even in old cultures on the Loeffler's medium the bacillary phase *a* was often non-viable, and on transplant to fresh media phases *b* and *C* often grew temporarily to the practical exclusion of the bacillary phase, as seen in figures 7 and 8. However, after twenty-four hours phase *b* began to dissociate the normal bacillary phase *a*, which thereafter completely dominated the picture. This reproduction could be observed directly under the

warm stage much after the manner of germination observed by Gardner (1925) with the "Y" forms of the dysentery group.

It should be perfectly clear then that any one ontogenetic phase of a culture may multiply almost to the exclusion of the other phases under conditions suitable for it, without mutation having occurred; but it should be equally clear if the multiplication is to be continued under a variety of conditions that the reorganizations incident to the sexual cycle must condition it, as indicated in the genetic origin of the mutant strains, K. A. 105 and K. A. 257.

Inasmuch as the K. A. strain is a diphtheroid it is of great interest to summarize briefly the work of others with the well known Park No. 8 diphtheria strain, since their collective observations point definitely to the existence of an ontogenetic cycle for this strain. Many years ago Dr. Anna Williams (1910) threw suspicion on the involutionary nature of the "branching involution forms," so-called, of this strain when she showed by direct observation that their metachromatic granules seemed to fuse before fission took place, and this fission she interpreted as a primitive sexual process. Moreover these branching forms were the only ones in the culture to show active growth and division under the condition of the experiment.

Just recently Martin, Loiseau and Gidon (1924) have noted that the same strain under partially anaerobic conditions yielded long filaments with lateral branches. Although this development begins after twenty-four hours and reaches its maximum by the eleventh or thirteenth day, the forms are still strongly Gram-positive, even after sixty days at 37°. It is significant that they consider these forms *not as evidence of degeneration*, but as normal developments under partially anaerobic conditions.

Again Heinemann (1917) working with this same strain noted that its almost complete loss of toxin producing power was correlated with a radically changed morphology. He found it to be reproducing in streptococcus and diplococcus form which bred true on glucose agar. Transplanted to Loeffler's medium the bacillary form of normal virulence was restored. It is obvious that this Park No. 8 strain undergoes important biologic alterations which are associated with its ontogenetic or pleomorphic

cycle and as such is strictly comparable to our K. A. strain, further evidence of whose heterogeneity will now be considered.

THE ORIGIN OF HETEROGENEITY IN BACTERIAL CULTURES

These experiments indicate clearly that the pleomorphic forms of a bacterial culture are *in a sense* independent entities capable of multiplication *as such* for a limited number of generations, at least. If—as will be shown—these phases have other physiological differences which emphasize their individuality, then we are forced to the conclusion that such cultures are in reality heterogeneous assemblages of individuals whose origin is the pleomorphic cycle.

In addition to the fact that these individual phases may reproduce as such, it is important to note that the mechanism of reproduction is not always the same. Phases *a* and *e* reproduce by transverse fission; phase *b* by branching, gonidial and zygospore formation (fig. 1, 6 and 7); and phase *C* by budding (figs. 8 and 9 at *a*). The latter being an ontogenetic phase, it proved impossible of course to isolate it, yet multiplication by budding could be observed directly under the microscope. Moreover the inoculation material contained relatively few of these circular forms (cf. fig. 1); yet in the transplant they dominated the morphologic picture (fig. 8) until they were overgrown later by the normal bacillary phase. The latter originated from the coarse branching phase *b* of figure 7. Here is definite proof of *reproductive heterogeneity* in a pure line culture.

The increase viability of phases *b* and *c* indicate cultural heterogeneity in respect to this character. As indicated, the origin of phase *a* from *b* and *c* prevented the cultures from dying out altogether. Old cultures of the original strain which did not contain these phases stained poorly, if at all. This was the case when the strain was grown on plain or blood agar instead of Loeffler's; but on the latter medium where the cultures were viable for many months, or even for more than a year, phases *b* or *c* were the only staining forms in the culture. Variant strain K. A. 191, representing phase *e*, maintains viability with little or no reproduction of the special phases on ordinary media or Loeffler's;

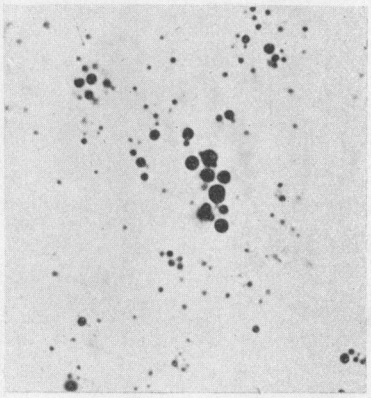

FIG. 2

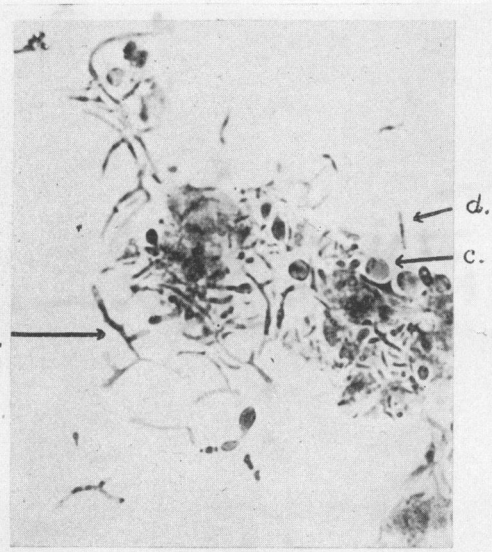

FIG. 7

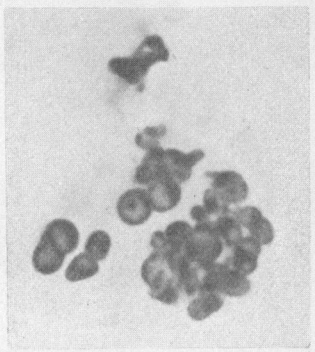

FIG. 8

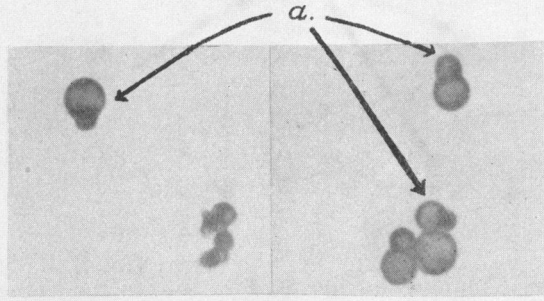

FIG. 9

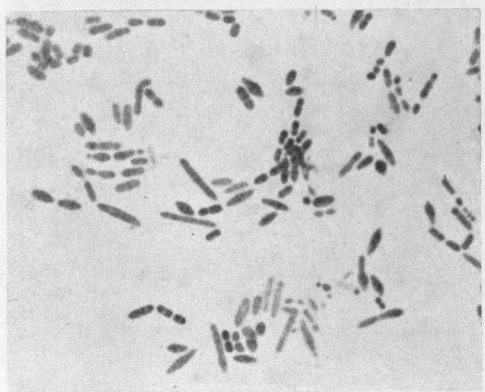

FIG. 10

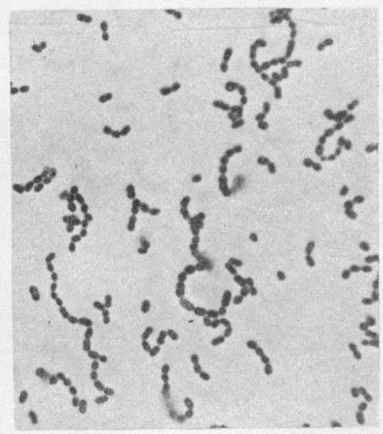

FIG. 11

yet on egg media these organisms frequently appear in fair numbers, showing that the capatiy for their formation had been suppressed but not entirely lost.

The variant strain K. A. 191 had acquired pigment formation and grew readily at 20°, which was not the case with the original strain. This temperature adaptation was suggestively associated with the gonidial phase *c*, since when these forms were present in an old culture it germinated readily at 37° but not at all at 20°, while old cultures which contained only the bacillary phase *a*, germinated readily at 20° but failed to do so at 37°. Such a fact might be of considerable importance for the initiation of infection with these organisms.

It will be recalled that the different phases of the ontogenetic or pleomorphic cycle reacted somewhat differently to the Gram stain. The coccoid and fungoid phases retained the Gram stain better than did the simple non-granular bacillary phase. It is not surprising then that the phylogenetic races should exhibit a corresponding variability.

The Gram amphophilism of the individual zygospores (phase *C*) is strikingly repeated in their lineal descendant, the coccus mutant strain K. A. 105 (fig. 5). Although today it would be called a frank Gram-positive coccus it is never quite free from the Gram-negative forms, which greatly increase in number when the culture is two or three days old.

They are small and appear to arise by budding from the larger Gram-positive forms. Even in younger cultures one-half of the coccus is frequently Gram-positive and the other half Gram-negative. The ontogeny of this coccus strain reveals under suitable conditions a succession of phases beginning with large coccoid forms, tetrads, and even at times bacillary forms (fig. 5). In short the cycle is reversed from that occurring with the pure line *bacillary* culture, but the interpretation is precisely the same. This strain produces a viridans type of hemolysis and many strains of *S. viridans* are as much diphtheroid in their morphology as coccus. That this interpretation holds just as nicely for the ontogeny of the cocci as for the bacilli we have experimentally shown in Study III of this series (Mellon, 1925c).

Figure 10 shows the morphology of single cell culture of an organism in a twenty-four-hour culture on a blood agar slant, which grows in ascitic fluid or broth as figure 11. One is a diphtheroid, the other a long chained streptococcus. The literature contains numerous instances of strains of this sort of which I shall quote but one, that of Ohlmacher (1902) who showed that streptococci and *B. coli* may germinate as practically pure rods or cocci, depending on the environment. The reversibility of the phenomenon caused a negative biologic evaluation to be assigned it by bacteriologists.

Its real significance we have experimentally demonstrated, first with the J strain (1917) and later with pure lines of the K. A. strain (1920). The actual mutation from diphtheroid to streptococcus that has been accomplished in these instances is a realization of the *potential phylogeny* represented by the ontogenetic phases of the culture. *It means, in short, that stabilization of these phases is a possibility, but only through a definite mechanism such as we have described.*

Again, the phenomenon of one phase of a culture producing acid and another phase alkali has already been set forth in Study II of this series (Mellon, 1925b). It represents one more aspect of heterogeneity that seems explained on these grounds. There is evidence, too, that alterations in virulence may often represent an aspect of a heterogeneity fundamentally no different from the others. In Study III of this series (Mellon 1925c) we have observed sudden acquisition of virulence in an alkaligenes strain that was definitely associated with a phylogenetic dissociation. Heinemann (1917) several years ago made a similar observation with the Klebs-Loeffler bacillus. This strain showed strikingly the reversible coccus and bacillary stages which could be correlated with differences in virulence. Latterly Amoss (1925) and Reimann (1925) show that pneumococci may be heterogeneous with respect to virulence, although they give no indication of the origin of that heterogeneity. It seems possible that this conception might have yielded much to the field of practical epidemiology had it not been restrained by the preconceptions to which I have referred.

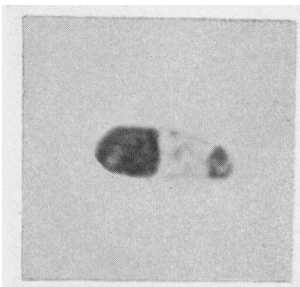

FIG. 12

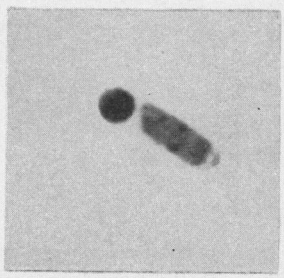

FIG. 13

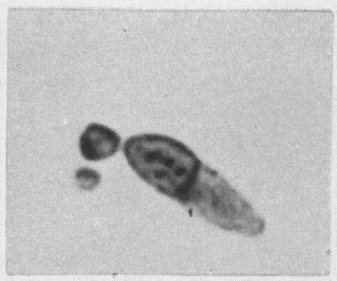

FIG. 14

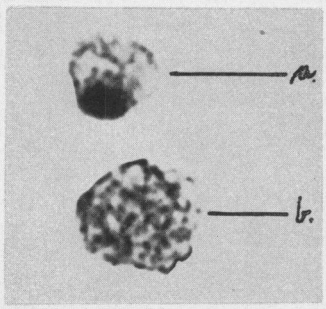

FIG. 15

FIG. 16

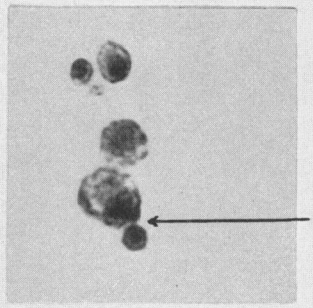

FIG. 17

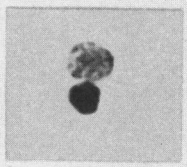

FIG. 19

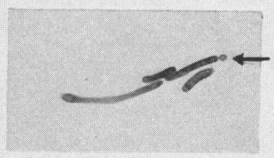

FIG. 20

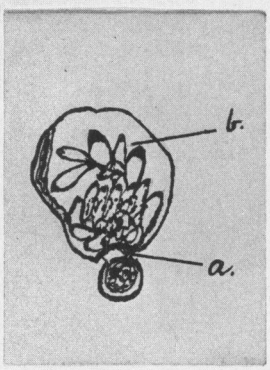

FIG. 18

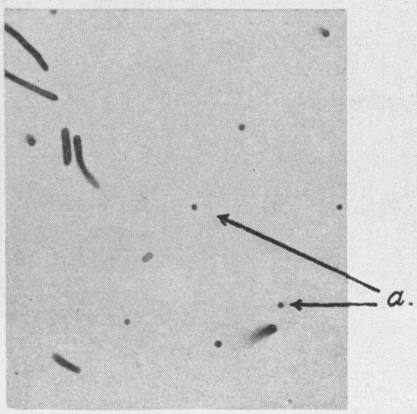

FIG. 21

(Mellon: Studies in microbic heredity)

ATYPICAL REPRODUCTION OF B. COLI

Inasmuch as this paper has dealt with the different methods of reproduction that may occur with different phases of a single culture, it seems desirable to include here a few observations on the capacity shown by *B. coli* to bud under suitable conditions. These conditions are described in detail on page 487 of Study I of this series (1925a) and the strain under consideration is the Nx one of that paper. The forms, in addition to being larger than normal as a result of the conditions under which they were grown, were stained intravitally which avoids fixation artifacts. Moreover they were magnified from 1500 to 3000 diameters, all of which accounts for their huge size.

In figure 12 we have a bipolar organism, and in figure 13 a unipolar one with early constriction of a terminal gonidial granule. In figure 13 no division wall is present as in strict fission, while in figure 14 the snaring off of a primary bud is followed by a similar process with a secondary bud. In figure 15 are two microgonidial forms which originated from the filaments that were common with this strain. Note typical early budding at *a*, with the process more advanced in figure 16 where constriction is beginning. In figure 17 at *a* the bud is about snared off and it is particularly noteworthy that the chromatin of the maternal cell participating in the division is thrown into a skein where twenty loops can be counted in the negative. It is almost a karyokinetic evolution of the nuclear matter. Diagrammatic representation of the same is seen in figure 18 where a secondary loop formation of the chromatin is seen at *b*. Note crater formation at *a*, typical for the germinating forms of this species. In figure 19 the bud is practically freed as the daughter cell containing the deeply staining chromatin.

The method of reproduction shown in the large forms of *B. coli* (figs. 12 to 14, inclusive) can be seen in other strains of coli where the bacilli are of normal size as they grow in ordinary broth. Figures 20 and 21 at *a* show this type of reproduction which represents an intermediate stage between true fission and budding. The resulting gonidia are seen at *a* in figure 21. Although the

latter usually elongate to bacilli they may occasionally continue to grow as a diplococcoid race of *B. coli*.

This is a confirmation of the work of Hort (1917) who observed directly under the microscope similar reproductions with *B. typhosus*. Again we see that the characterization of bacteria as fission fungi must be regarded as only relatively true.

DISCUSSION

The half knowledge of these essential points bequeathed us by tradition has largely contributed to the confused and dogmatic position taken by certain sections of orthodox bacteriology in regard to them. The doctrines of monomorphism have developed no intelligent conception of the manner in which new types (developmental stages) might be *potentially present* in perfectly pure line cultures. In the past, experiments that have realized this potentiality in the dissociation of new types out of the pleomorphic cycle, have usually been prejudged on the ground that pleomorphism among the bacteria had no meaning, and the new type must have persisted *as such* in the original culture.

In recent years practical considerations in respect to the agglutination reaction leave little doubt that many bacterial cultures are heterogenous; yet so great has been the obeisance to the *involution* anachronism that it is not yet realized that the real key to the origin of this heterogeneity is inextricably associated with the "involution" forms themselves. In short, heterogeneity is the physiological counterpart of bacterial pleomorphism, and as such is a proof that the latter has important biologic connotations.

The question arises whether we shall consider as mutants the new types that may at times arise in association with sexual reorganization. To Enderlein (1925) the physiologic and cultural changes that he has shown to result from sexual reorganization in *B. cholera* constitute a new "growth generation" and cannot be considered a mutation. On theoretical grounds he inclines to the view that most of our bacterial mutants, so-called, fall in the same class. It is my opinion that these rejuvenation changes need not preclude the occurrence at times of more far reaching

ones in the same anlagen, i.e., in the gonidia or zygospore. It appears to be largely a matter of the extent of the reorganization which their environment permits to occur in them. It would be difficult to assert that the reorganization resulting in slight changes is *fundamentally* different from that producing more far reaching changes of character.

Certainly when one is able to derive organisms as different culturally, morphologically and serologically as are the strains under consideration, it would be difficult to know what to consider mutants if these are not so regarded. With no disposition to take a dogmatic view of the situation I must tentatively regard the *stabilized* stages in the phylogeny of the organisms as variants or mutants if the new characters are *constant.* If the organism gradually reverts as it ages I would then regard the "new" organism as a "growth generation."

FERMENTATION REACTIONS

The cultures were incubated for from seven to ten days at 37°C. in sugar free broth to which 1 per cent of the various sugars and bromothymol blue were added to a slight greenish tint. Good growth was obtained in all tubes. The pH of the uninoculated controls without any sugar was 7.0; of the inoculated controls 7.0 to 7.02. The readings were colorimetric except in case of doubt when they were checked with the hydrogen electrode. They were made after seven days at 37°C. and again after seven days more at 20°C.

The tubes which produced acid in from seven to ten days usually produced alkali after that period if they had not already shown it. Salicin was somewhat of an exception with the K. A. 257, inasmuch as the formation of acid was rather slow, but in fourteen days it was very definite, both by titration and colorimetrically. It will be seen that this was the only qualitative fermentation difference between K. A. 95, K. A. 191 and K. A. 257. With K. A. 105, however, mannitol, dextrin, lactose and probably insulin were fermented, which is not the case for the other three strains.

SEROLOGIC REACTIONS

Immune sera were developed in rabbits by intravenous inoculation of killed cultures of the various strains which had been isolated from a single cell, and grown on plain agar. From four to six inoculations were given over a course of two weeks. Sera were tested from seven to ten days later and if of sufficiently high titre the animals were bled and the sera put in the ice chest. Before inoculation the rabbit's sera were tested with all strains, which served as normal control sera. These were repeated when the experiments were done. The tubes were incubated two hours at 37° and read, then put in the ice chest over night and read a second time. After twenty-four hours more at 20° they were again examined.

The adsorption technique was the same as detailed in Study III of this series. K. A. 95 antiserum was developed at a time when the original bacillus was not pigmented and did not grow very luxuriantly. K. A. 191 antiserum, was made a couple of years later when the organism had acquired pigment formation, grew more luxuriantly and had lost to a large extent its capacity to form coccoids.

In the table, C indicates that agglutination is complete; 3+, 2+ and 1+ indicate a progressively lessened amount of it; − is negative; a blank space indicates no test.

Analysis of tables 2 and 3

From tables 2 and 3 it is clear that K. A. 191 and K. A. 257 agglutinate to full titre in each other's sera. Since K. A. 191 agglutinates to full titre in K. A. 95 serum it is probable that the reverse reaction would have been similar had it been possible to carry it out. On the other hand there is no evidence of cross agglutination between the coccus K. A. 105 and any of the bacillary strains, using the *usual technique.*

However, in Studies III and IV of this series (Mellon, 1925a and 1926) we have shown that it is possible at times to detect the presence of small amounts of group agglutinins by employing a temperature of 20° instead of 37° for incubation. Accordingly

TABLE 2

Agglutination reactions

ANTIGEN	TITRE OF THE HOMOLOGOUS SERA									NORMAL SERUM			NaCl 0.85	ANTISERUM
	1–20	40	80	160	320	640	1280	2500	5009	1–20	40	80		
K. A. 95	C	C	C	C	C	3+	–	–	–	–	–	–	–	K. A. 95
K. A. 257	C	C	C	C	C	C	C	3+	–	–	–	–	–	K. A. 257
K. A. 191	C	C	C	C	C	C	3+	–	–	–	–	–	–	K. A. 191
K. A. 105	C	C	C	C	3+	–	–	–	–	–	–	–	–	K. A. 105

TABLE 3

Cross agglutination reactions

ANTIGEN	1–20	40	80	160	320	649	1280	2500	5000	NORMAL SERUM			NaCl 0.85	ANTISERUM
										1–20	40	80		
K. A. 191	C	C	C	C	2+	–				–	–		–	K. A. 257
K. A. 105	1+	1+	–	–	–	–				–	–	–	–	K. A. 257
K. A. 95*													–	K. A. 257
K. A. 257	C	C	C	C	C	C	C	C	–	–	–	–	–	K. A. 191
K. A. 105	1+	1+	1+	–	–	–	–			–	–	–	–	K. A. 191
K. A. 95*													–	
K. A. 257	±1	±1	–	–	–	–	–			1+	–	–	–	K. A. 105
K. A. 191	±1	±1	±1	–	–	–	–			1+	–	–	–	K. A. 105
K. A. 95	±1	±1	–	–	–	–	–			–	–	–	–	K. A. 105
K. A. 105	−1	–	–	–	–	–	–			–	–	–	–	K. A. 95
K. A. 191	C	C	C	C	C	3+				–	–	–	–	K. A. 95
K. A. 257†													–	K. A. 95

* Not done because K. A. 95 did not regenerate when the variant K. A. 191 developed from it.

† Not done because K. A. 95 serum was exhausted at the time variant K. A. 257 was dissociated.

this was done. When K. A. 105 was run against K. A. 257 serum at 24° no agglutination could be detected, but K. A. 105 vs. K. A. 191 serum showed a definite flocculation in six tubes at the end of one hour.

Although the flocculation could be readily seen with a hand lens at the end of twenty-four hours there was little, if any, increase in it, and actual precipitation was scarcely noticeable. No agglutination occurred with the normal serum of K. A. 191 at 24°. It is desirable to employ a temperature of 20° rather than 24° as the results are usually sharper. At the time the tests were done the room temperature was 24° and it was not convenient to use 20°, especially when the adsorption test was conclusive.

Analysis of table 4

As seen in table 4 the antisera of K. A. 257 and K. A. 191 reciprocally adsorb to exhaustion the homologous agglutinins. K. A. 191 and K. A. 257 do not agglutinate in K. A. 105 serum even with the 20° technique, and the adsorption results are negative. K. A. 105 does not agglutinate in K. A. 257 serum, neither does it adsorb any agglutinins for K. A. 257 from this serum. On the other hand at 24° K. A. 105 does show partial agglutination in K. A. 191 serum and on adsorption reduces the titre of the latter from a 3+ in a dilution of 1280 (table 2) to a ±1 in a dilution of 640.

This result is of striking interest, since it appears that a pigmented diphtheroid bacillus not affecting blood agar produces group agglutinins for a frank diplococcus, non-pigmented, but producing a viridans type of hemolysis on blood agar. The sugar reactions of the two strains are also divergent. Although the reduction in titre of the serum is quantitatively not striking, it is appreciable, definite and constant for this serum.[2] The fact

[2] Several other animals were immunized with K. A.-191. These sera were adsorbed with K. A.-105 antigen and the reactions with the homologous strain varied from demonstrable ones to one that is practically inappreciable. These differences in reaction in different animals where small amounts of group agglutinin are concerned find detailed explanation in Study No. III of this series (loc. cit.).

TABLE 4

The results of adsorption

ANTIGEN	1–20	40	80	160	320	640	1280	2500	NaCl 0.85	SERUM
K. A. 257	–	–	–	–	–	–			–	K. A. 191 c Ant. 257*
K. A. 191	–	–	–	–	–	–			–	K. A. 191 c Ant. 257
K. A. 105	–	–	–	–	–	–			–	K. A. 191 c Ant. 257
K. A. 191	–	–	–	–	–	–			–	K. A. 257 c Ant. 191
K. A. 257	–	–	–	–	–	–			–	K. A. 257 c Ant. 191
K. A. 105	–	–	–	–	–	–			–	K. A. 257 c Ant. 191
K. A. 105	–	–	–	–	–	–			–	K. A. 191 c Ant. 105
K. A. 257	C	C	C	C	C	C	C	3+	–	K. A. 191 c Ant. 105
K. A. 191	C	C	C	C	C	±			–	K. A. 191 c Ant. 105
K. A. 105	–	–	–	–	–	–			–	K. A. 257 c Ant. 105
K. A. 257	C	C	C	C	C	C	C	3+	–	K. A. 257 c Ant. 105
K. A. 191	C	C	C	C	±				–	K. A. 257 c Ant. 105
K. A. 105	C	C	C	C	C	1+	–			K. A. 105 c Ant. 257
K. A. 191	–	–	–	–	–	–	–			K. A. 105 c Ant. 257
K. A. 105	C	C	C	C	C	2+	–			K. A. 105 c Ant. 191
K. A. 191	–	–	–	–	–	–	–			K. A. 105 c Ant. 191

* c stands for "adsorbed with;" Ant. for "antigen."

that there is no suggestion of such a result with strain K. A. 257 (which agglutinates and adsorbs reciprocally with K. A. 191) may mean that it is not precisely correct to base serologic identity on reciprocal adsorptions. It is probably beyond the realm of possibility to say that the antigenic complexes of two unknown strains are identical, since it would be impracticable to test out the antisera of each with a host of other strains.

The experiment is quite revealing in another direction, showing as it does that a failure of two culturally and morphologically diverse organisms to cross agglutinate does not preclude their genetic origin from a common ancestor. It is pertinent to refer to a study made by us several years ago (1919b) in which we showed that the spore and vegetative phases of the same microörganism yielded two distinct agglutinogens, from which were produced antisera that were not reciprocally adsorbed to any appreciable extent.

Strain K. A. 105 was dissociated six years ago; strain K. A. 257 was split off three years ago, and K. A. 191 four years ago. They have been quite constant in every respect since this dissociation. In order further to control this experiment an attempt was made to adsorb K. A. 191 serum with a hemolytic streptococcus (A-12) isolated at autopsy. The result was completely negative, so that we have no evidence that the cross adsorption of K. A. 105 for K. A. 191 serum could be referred to some nonspecific adsorbing factor characteristic of the streptococcus group as a whole.

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CONCLUSIONS

1. The biogenetic law of Haeckel is capable of application to the genetic history of bacteria.

2. The various phases of the pleomorphic cycle represent the ontogeny of a strain; when the phases are stabilized through mutation they collectively represent the phylogeny of this strain.

3. The bacterial classifications that *widely separate* the cocci from the bacilli do not find genetic support, irrespective of the practical value of such artificial division.

4. Group and specific agglutinogens have been demonstrated between stable races of bacilli and cocci.

5. Complete serologic reciprocity has been shown for two races of bacilli, morphologically and culturally quite diverse.

6. Considerable biologic diversity has been shown to exist among the various phases of the pleomorphic cycle. On this fact can be appropriately based the heterogeneity of pure line cultures.

REFERENCES

AMOSS, H. L. 1925 The composite nature of a pure culture of a virulent pneumococcus. Jour. Exper. Med., **41**, 649–662.

ENDERLEIN, GUNTHER 1925 Bakterien-Cyclogenie Prolegomena zu untersuchungungen über Bau, geschlechtliebe und ungeschlectliebe Fortpflanzung und Entwicklung der Bakterien. Walter de Gruyter & Co., Berlin and Leipsig.

GARDNER, A. D. 1925 The growth of branching forms of bacilli ("three-point multiplication"). Jour. Path. and Bact., **28**, 189–194.

HEINEMANN, PAUL 1917 The morphology of a strain of *B. diphtheria*. Jour. Bact., **2**, 361–363.

HORT, EDWARD C. 1917 The life history of bacteria. Brit. Med. Jour., May 5, 1917, 571–575.

MARTIN, LOUIS, LOISEAU, GEORGES AND GIDON, VICTOR 1924 Production des formes ramifiées du *Bacille diphtérique* (production of branched forms of *B. diphtheriae*). Compt. rend. Soc. de biol., Par., **91**, 332–334. Abst. in Abst. Bact., **9**, June, 1925, 245.

MELLON, RALPH R. 1917 Study of the diphtheroid group of organisms with special reference to their relation to the streptococci. Jour. Bact., **2**, 81–107, 269–307, 447–500.

MELLON, RALPH R. 1919 Contribution to the bacteriology of a fuso-spirillary organism, with special reference to its life history. Jour. Bact., **4**, 505–538.

MELLON, R. R., AND ANDERSON, L. M. 1919 Immunologic disparities of spore and vegetative stages of *B. subtilis*. Jour. Immun., **4**, 203–208.

MELLON, RALPH R. 1920 The life cycle changes of the so-called *B. Hodgkini*, and their relation to mutation changes in this species. Jour. Med. Res., **42**, 61–76.

MELLON, RALPH R. 1922 Spontaneous agglutinability of bacteria in relation to variability and to the action of equilibrated solutions of electrolytes. Jour. Med. Res., **43**, 345–367.

MELLON, RALPH R. 1925a Studies in microbic heredity. I. Observations on a primitive form of sexuality (zygospore formation) in the colon-typhoid group. Jour. Bact., **10**, 481–501.

MELLON, RALPH R. 1925b Studies in microbic heredity. II. The sexual cycle of *B. coli* in relation to the origin of variants with special reference to Neisser and Massini's *B. coli mutabile*. Jour. Bact., **10**, no. 6, 579–588.

MELLON, RALPH R., AND JOST, ELIZABETH 1925c Studies in microbic heredity. III. The hereditary origin of group and specific agglutinogens among the colon-alkaligenes organisms. Jour. Immun., In press.

MELLON, RALPH R., AND GRENQUIST, ERNST 1926 Studies in microbic heredity. IV. Observations on group agglutinins in specific sera with the technique of cataphoresis. (In press—Jour. of Immunology.)

OHLMACHER, A. P. 1902 Observations upon the morphological variations of certain pathogenic bacteria. Jour. Med. Res., **7**, 127–128.

PARK, WILLIAM HALLOCK, AND WILLIAMS, ANNA W. 1910 Pathogenic microorganisms including bacteria and protozoa. Lea & Febiger, New York and Philadelphia, p. 18.

REIMANN, H. A. 1925 Variations in specificity and virulence of pneumococci during growth in vitro. Jour. Exper. Med., **41**, 587–600.