ANTIGEN VARIATION IN PARAMECIUM AURELIA, VARIETY 1

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IN recent years the importance of the cytoplasm as a determinant of hereditary characters has become increasingly apparent. It has even been claimed that the cytoplasm contains genetic units as autonomous and specific as the nuclear genes, though such claims have been much disputed. Paramecium is exceptionally suitable for the investigation of this problem, and thanks to the work of SONNEBORN and his colleagues a considerable amount of information concerning the effect of cytoplasmic materials on certain characters of the organism, such as the "killers" and the antigens, has been obtained.

Investigation of these phenomena has hitherto been concentrated on the "B" group of varieties of *P. aurelia* (mainly variety 4), and the evidence at first seemed to show that cytoplasmic heredity was a feature of these varieties only. By contrast members of the "A" group (including variety 1) showed only Mendelian heredity.

The object of the present study was a more detailed analysis of the genes concerned with antigen formation in a number of stocks of *P. aurelia*. For this purpose, variety 1 was chosen, because some early observations of SONNEBORN (1943) had indicated a straightforward, Mendelian, segregation of antigen types in this variety. It was hoped that a more complete knowledge of the genic system would make it easier to place in its correct perspective the influence of the cytoplasm, about which considerable information is now to hand in variety 4. At the same time it was hoped to reconcile the apparent discrepancies in the behavior of the two groups of varieties, for, as SONNEBORN (1945) points out, it is hardly likely that two so nearly related groups of organisms would contain totally distinct hereditary mechanisms.

MATERIALS AND METHODS

Four stocks of *P. aurelia* were used in these studies. They were stocks 90, 60, 41 and 61, all belonging to variety 1 and mating freely together. They had been collected from various localities in N. America and were kindly supplied by DR. SONNEBORN.

Techniques for culturing, crossing, induction of autogamy and preparation of antisera were substantially the same as those previously described (SONNEBORN 1950a).

RESULTS

Variation within stocks

Previous work with variety 4 (SONNEBORN 1950b) had shown that each stock, derived from a single homozygous individual, was capable of giving rise to a series of antigen types, and that this intra-stock variation was caused by some mechanism other than the substitution of one gene by another. In variety 1, it was very soon evident that a similar system occurred. For example, an antiserum was prepared by injecting paramecia of stock 90 into a rabbit, and designated anti-90G. At a dilution of 1/1500 this antiserum was effective in immobilizing paramecia of the same type as those injected. A small proportion of paramecia of the same culture were however completely resistant to the immobilizing action of the antiserum. Cultures of these resistant animals were then grown, injected into a rabbit, and a new antiserum, anti-90D, prepared. This second antiserum immobilized paramecia of the second type (90D), but not those of the first type (90G). By similar procedures it was found that stock 90 could develop yet other antigen types. In table 1, the interactions between three commonly formed types (D, G, S) of stock 90 with the three corresponding antisera are shown.

TABLE 1

Immobilizing titres of 3 antisera of stock 90. The dilutions shown are those required to immobilize a few paramecia in 2 hrs. Dilutions below 1/50 are not considered.

Serum	Paramecium types		
	90D	90G	90S
Anti-90D	1/1500	....	....
Anti-90G	1/50	1/1500	....
Anti-90S	....	1/50	1/1500

The weak cross-reactions shown in certain combinations may be due to the presence of a small proportion of animals of undesired type in the injected material; or, alternatively, to the presence of a heterologous antigen at some site other than the surface of the paramecium (SONNEBORN, unpublished) or to some other cause. In general, cross-reactions between different types of the same stock were remarkably weak.

Effect of growth at varying temperatures

Paramecia of stock 90, when grown at 25°C, were predominantly of type G, but a small proportion of them were of types D or S. Growth at 29° or higher resulted in the production of predominantly type D animals, while at 18° type S could be produced. Transformation of one type to another as a result of changing temperature occurred in a regular manner, according to conditions. For example, a certain sample of 90G animals, when grown at 29° with excess food, transformed to 90D after approximately 50 fissions (or

ten days); if growth was at 33°, this transformation occurred after ten fissions (or two days); while growth at 36° produced transformation in less than five fissions (one day). The actual change of type from 90G to 90D was always quite sudden, occupying the time of only about 2 fissions, and during this short transitional phase the animals reacted simultaneously to both anti-90G and anti-90D sera. During the preceding period of growth at a new temperature, however, no perceptible change occurred in the 90G animals; they remained exactly the same as they had been when grown at 25°. The paramecia are thus able to maintain either one or other of these two antigens, but not both simultaneously.

The transformation from 90G to 90D was readily reversible by growing the 90D animals at 25° with excess food. Under these conditions 90D reverted to 90G in about 11 fissions (three days).

Variation between stocks

All the four stocks studied (together with 11 others not described here) behaved similarly, though not identically, in their response to varying temperatures. All produced a characteristic series of antigens. In all stocks the antigens formed at 29°–33° corresponded to a greater or less degree to type D of stock 90; those formed at 25° corresponded to type G of stock 90, and those formed at 18° corresponded to type S of stock 90. This regular correspondence between the three types formed by each of the four stocks was revealed by both serological relationships and genic relationships, as will be shown.

Data showing the serological relationship between corresponding types in different stocks are given in table 2. (Non-corresponding types showed little or no serological relationship.) It will be seen that some "corresponding" types were indistinguishable by the serological tests used, *e.g.*, 90S, 60S and 41S, or 61D and 90D; others were rather closely related yet clearly not identical, as shown by cross-reactions, *e.g.*, 60D and 41D; while some were so distinct that they showed practically no cross-reactions at all, *e.g.*, 61D and 60D, or 90G and 60G. For this reason, in a former publication (BEALE 1951), separate letters were applied to certain types. (The present 90D = the former 90Y, 90G = 90Z, 60D = 60W, and 60G = 60X.) The simplified nomenclature adopted here is based partly on the gene analysis, presented below, and partly on the homologies between certain of the D and G types found here in variety 1 with some of the D and G types already known in variety 4 (SONNEBORN, WHALLON and BEALE, unpublished).

Some types were much more easily obtained than others. For example, in stock 90, type 90G was invariably obtained by growth at 25°, but at 18° the type 90S was formed only sporadically and frequently transformed back to 90G. In order to obtain a pure culture of 90S it was necessary to grow the animals at 18° and kill unwanted 90G animals with homologous antiserum. In stock 41 however the situation was reversed. At 18° 41S was readily produced, but at 25° 41G appeared only occasionally and often transformed to 41S or 41D. Thus the *stability* of the corresponding types of different stocks

TABLE 2

Immobilizing titres of 12 antisera, each tested against four "corresponding" types of paramecia.

Serum		Paramecium types formed at 29°–33°			
No.	Description	90D	60D	41D	61D
45	anti-90D	1/1500	1/50	1/50	1/1500
72	anti-60D	....	1/1500	1/500	....
23	anti-41D	....	1/250	1/1000	....
60	anti-61D	1/1000	....	....	1/1000
		Paramecium types formed at 25°			
		90G	60G	41G	61G
64	anti-90G	1/1500	....	1/150	1/100
7	anti-60G	....	1/500	....	....
32	anti-41G	....	....	1/1500	1/500
66	anti-61G	1/50	....	1/500	1/1500
		Paramecium types formed at 18°			
		90S	60S	41S	61S
63	anti-90S	1/1500	1/1500	1/1500	....
50	anti-60S	1/1500	1/1500	1/1500	1/150
20	anti-41S	1/1500	1/1500	1/1500	1/50
54	anti-61S	1/50	1/50	1/50	1/1000

is by no means the same. It is difficult to specify exactly the stability, or the optimal conditions for a given type, but in general the following were found to be rather unstable or to require a narrow range of conditions: 90S, 60G, 41G, 61G. All the other types described were readily formed at the appropriate temperatures.

The *order* of the types with respect to temperature was however remarkably uniform, not only with the four stocks described here, but also with all other stocks examined.

Gene differences between stocks

Crosses were made between animals of each of the four stocks in various combinations, and the F_1 animals so obtained made to pass through autogamy. Segregation of antigenic types in the exautogamous F_2's was observed, and an analysis made of the gene differences between the four stocks. In all these experiments the parental cultures were first passed through autogamy to ensure their complete homozygosity.

F_1's. Hybrids between any two stocks were found to develop, in about the fifth cell generation after conjugation and thereafter, a mixture of antigens characteristic of the two parents, that is, any F_1 animal could be immobilized by two kinds of antibodies, one of which would affect only one parent and the second the other parent. The delay of approximately five fissions between

introduction of a new nucleus at conjugation and the manifestation of some of its genes, was presumably connected with the time necessary for the construction of a new macronucleus and the gradual dispersal of the products of the old macronucleus. A similar period of delay was found by SONNEBORN (1950c) for the manifestation of a gene distinguishing stock 29 from stock 51 of variety 4. Table 3 shows the antigens formed in various hybrids, grown at the three temperatures.

TABLE 3

Antigenic types of F_1 hybrids

Parent stocks	29°–33° group	25° group	18° group
90 × 60	90D + 60D	90G + 60G	S
90 × 41	90D + 41D	90G + 41G	S
90 × 61	D	90G + 61G	90S + 61S
60 × 41	60D + 41D	60G + 41G	S
60 × 61	60D + 61D	60G + 61G	60S + 61S
61 × 41	61D + 41D	61G + 41G	61S + 41S

These results are arranged for convenience in columns headed by the temperatures at which the types shown were most readily formed. Some of the types however were unstable (like some of the parents), and transformed into types more characteristic of other temperatures, *e.g.*, the F_1 between stocks 60 and 41 at 25°, shown as 60G + 41G, often transformed even at 25° to 60D + 41D.

The F_1's showed the following features: (1) Whenever two parental stocks could develop distinguishable antigens in a given temperature group, antigens characteristic of both parents could be detected in the F_1; but the immobilizing reactions between F_1 animals and one or other of the parental antisera were usually less strong than the immobilizing reactions between the parental animals themselves and their respective homologous antisera. It may therefore be concluded that the amount of any one antigen in the F_1 was less than in the parent, though since the F_1 contained two antigens the total amount may well have been the same. Frequently the F_1's resembled one parent much more closely than the other. (2) The F_1 animals never contained, except as a temporary condition during transformation, mixtures of antigens characteristic of different temperature groups, *e.g.*, the F_1 90 × 60 contained either 90G + 60G or 90D + 60D, but not 90G + 60D or 90D + 60G etc.

F_2's. The exautogamous F_2 families showed segregation of the antigen types characteristic of the parent stocks. For example, from the cross 90 × 60 there appeared in the F_2: at 18°—type S; at 25°—types 90G and 60G in a ratio of 1 : 1, and at 33°—types 90D and 60D in a ratio of 1 : 1. In a given temperature group, no antigenic types appeared which did not occur in one or other of the parent stocks, and when the parents differed in the antigens produced at a given temperature, the two types reappeared in the F_2 in equal numbers. The results are summarized in table 4.

TABLE 4

Segregation of antigen types in ex-autogamous F_2 families

Cross	29°–33° group			25° group			18° group		
90 × 60	90D 38	60D 36	Dead 176	90G 95	60G 82	Dead 7			
90 × 41	90D 95	41D 91	Dead 6	90G 142	41G 138	Dead 94			
60 × 41	60D 98	41D 104	Dead 65	60G 57	41G 47	Dead 193			
60 × 61	60D 111	61D 112	Dead 206	60G 36	61G 36	Dead 21	60S 28	61S 27	Dead 37
61 × 41	61D 138	41D 142	Dead 89						

The wide fluctuations in proportions of "dead" clones shown in table 4 are due to the fact that some of the types could be determined at five fissions after autogamy, and where this was possible there was very little mortality, but others had to be grown for prolonged periods at certain temperatures in order to bring about transformation to the desired temperature group, and when this was done many clones died out. Such a high mortality in F_2's involving diverse stocks has been reported previously by SONNEBORN (unpublished) and DIPPELL (1950). Fortunately the ratios obtained here are not disturbed, even with 50 percent or higher mortality.

Each of the viable F_2 clones shown in table 4 was grown at various temperatures, and it was then found that they could show a recombination of parental characters. For example, in an F_2 from the cross 90 × 60, there appeared, not only the two parental classes (90G at 25°, 90D at 29°–33°; and 60G at 25°, 60D at 29°–33°), but also two new classes (90G at 25°, 60D at 29°–33°; and 60G at 25°, 90D at 29°–33°). The two new classes occurred in approximately the same numbers as the two non-recombination classes.

TABLE 5

F_2s showing recombination of two pairs of antigen types

Cross		Old classes		Recombination classes		Dead
90 × 60	Formed at 29°–33° → Formed at 25° →	90D 90G	60D 60G	90D 60G	60D 90G	
		20	17	18	19	118
90 × 41	Formed at 29°–33° → Formed at 25° →	90D 90G	41D 41G	90D 41G	41D 90G	
		31	39	53	48	213
60 × 41	Formed at 29°–33° → Formed at 25° →	60D 60G	41D 41G	60D 41G	41D 60G	
		32	22	22	24	197

TABLE 6

F_2 from the cross 60 × 61, at three temperatures

	Old classes		Recombination classes						Dead
Formed at 29°–33° →	61D	60D	61D	61D	61D	60D	60D	60D	
Formed at 25° →	61G	60G	60G	61G	60G	61G	60G	61G	
Formed at 18° →	61S	60S	60S	60S	61S	61S	61S	60S	
	5	5	6	7	10	6	6	10	37

Data on this subject are presented in table 5. There are signs of significant deviations from the expected 1: 1 : 1 : 1 ratios, but this is not surprising in view of the high mortality and the instability of certain types.

A similar recombination of antigenic characters occurred in all the other F_2 families. Stocks 60 and 61 differ markedly in the antigens they produce in all three temperature groups. Consequently the F_2 derived from a cross between these two stocks permits the detection of eight classes, of which two are the old ones and six are new, recombination, classes. Such an F_2 was analyzed, and the results are shown in table 6. Though the numbers in each class are small, representatives of all eight classes were obtained, as shown.

The results set out in tables 4, 5 and 6 can best be interpreted according to the following genetical system. Each of the four stocks contains genes at three separate loci—*g*, *d* and *s*. Only one of these gene-loci is normally effective in controlling the phenotype at a given time. Genes at the *g* locus are manifest in paramecia grown at 25°, genes at the *d* locus at 29°–33°, and genes at the *s* locus at 18°. The different stocks each have their characteristic alleles at each of the three loci, as indicated in table 7.

Those alleles which at present cannot be distinguished, namely the *s* alleles of stocks 90, 60 and 41, and the *d* alleles of stocks 90 and 61 are provisionally written without suffixes. There are therefore at present four distinct alleles at the *g* locus (g^{90}, g^{60}, g^{41}, and g^{61}), three at the *d* locus (d^{60}, d^{41} and *d*), and two at the *s* locus (s^{61} and *s*). None of these genes is completely dominant over any of its alleles, though some are nearly so.

Data so far obtained show no indication of linkage between any of the three loci, though the numbers obtained would not reveal loose linkages.

TABLE 7

System of antigen-determining genes in four stocks.

Stock	Antigens			Genes
	29°–33°	25°	18°	
90	90D	90G	90S	$d\ g^{90}\ s$
60	60D	60G	60S	$d^{60}\ g^{60}\ s$
41	41D	41G	41S	$d^{41}\ g^{41}\ s$
61	61D	61G	61S	$d\ g^{61}\ s^{61}$

The role of the cytoplasm

As described previously, there may be a delay of 50 or more fissions between transference of a culture to a new temperature and the onset of transformation from one antigen type to another. The occurrence of this delay makes possible the crossing of types which are stable at different tempera-

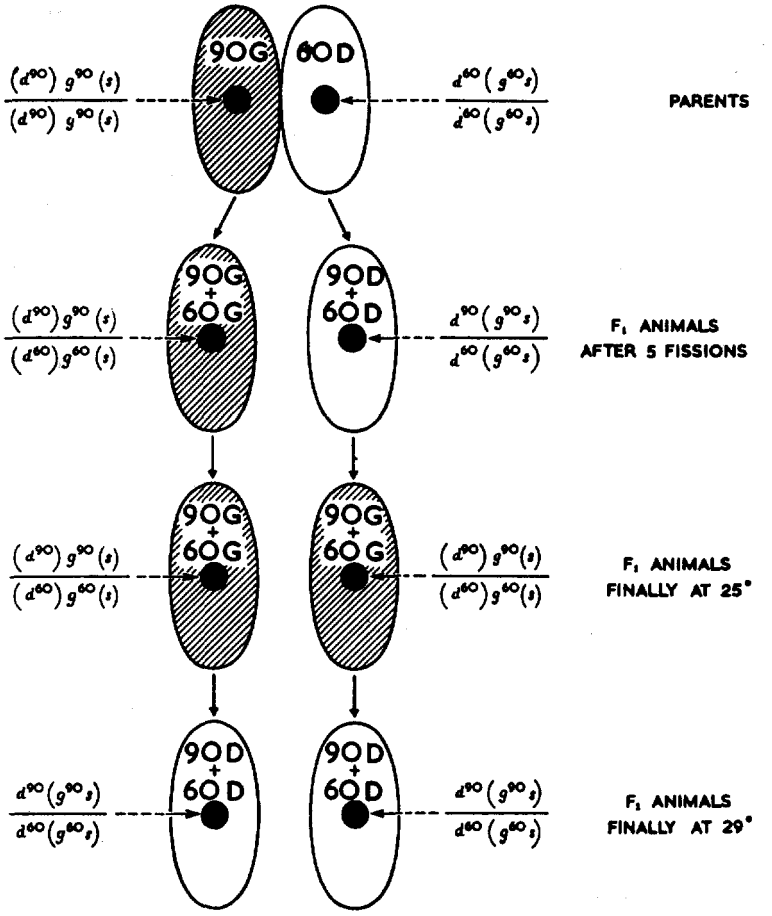

FIGURE 1.—The consequences of a cross between paramecia of the types 90G and 60D. The antigens are shown as large figures and letters and the genes as small italics. Genes which are not expressed are enclosed in brackets. Cytoplasm in the "25°" state is shown shaded; cytoplasm in the "29°" state is left clear.

tures, *e.g.*, 90G × 60D or 90D × 60G. Such crosses were made and the results are illustrated in figure 1. Considering the cross 90G × 60D it was found that after five fissions, those descendants which had received their cytoplasm from the 90G parent developed the antigens 90G + 60G, while those which had received their cytoplasm from the 60D parent developed the antigens 90D +

60D. If growth of the F_1 animals was continued at 29°, all the progeny finally came to have the antigens 90D + 60D, no matter what had been the parental cytoplasm; continued growth of the F_1 animals at 25°, however, eventually produced only 90G + 60G types.

These results show that the cytoplasm of the 90G animals is of such a nature as to permit the expression, not only of the gene g^{90}, but also of g^{60}. The cytoplasm of the 60D animals favors the expression of d^{90} and d^{60}. This property of the cytoplasm is transmitted for varying lengths of time to the descendants, but can be changed by varying the temperature.

It is therefore concluded that the action of varying temperatures on the expression of the genes at the three loci *g, d* and *s*, is effected through a modification of the cytoplasm. Considering again the cross 90G × 60D at 29°, it is to be noted that the nucleus from the 60D parent contains the gene g^{60} which is not expressed; but a nucleus from this parent migrates into the cytoplasm of the 90G parent and there contributes to the formation of a new macronucleus. The g^{60} genes in this new macronucleus are at once expressed, notwithstanding the fact that there has been no change of temperature, which is still 29°. What is necessary is that the cytoplasm into which the gene g^{60} is introduced should at some time previously have been brought to the "25°" state.

These conclusions have been confirmed by a number of other crosses. For example, 60G was crossed with 41D, and yielded, after five fissions at 25°, animals containing 60D + 41D if their cytoplasm was derived from the 41D parent, and 60G + 41G if their cytoplasm was derived from the 60G parent. In all such experiments it was found that the state of the cytoplasm determined which gene-loci were to be expressed, and that members of a series of alleles were favored by the same kind of cytoplasm.

Thus the earlier view of SONNEBORN (1948) that antigenic variations within a stock of variety 4 were cytoplasmically controlled, but that individual antigens could only be developed in the presence of particular genes, is strikingly confirmed here in variety 1.

DISCUSSION

From the preceding experiments, the following conclusions are drawn:

1. Each stock of *P. aurelia*, variety 1, contains a number of genes at different loci, corresponding to each of the antigens produced. Three such loci (*g, d, s*) have so far been identified. It is likely that there are several more.

2. Each stock has its characteristic alleles at each of these loci, though some alleles may be common to several stocks.

3. Within a homozygous stock only one immobilizing antigen is revealed at a time, except as a temporary phenomenon when a paramecium is transforming from one type to another. Consequently, genes at only one of the three loci can come to full expression at a given moment. There is a mutual exclusion of the products of non-allelic genes.

4. In heterozygotes, two antigens are regularly formed in the same animal, though each one separately in lesser amount than in the appropriate homozygote.

5. Which of the loci *d, g, s* will be expressed depends upon the state of the cytoplasm.

6. The state of the cytoplasm may be modified by varying the temperature of the external environment, though such modification need not occur immediately the organism is placed in the new environment. There may be a delay of 50 or more fissions. The change of state, however, when it does occur, takes place rapidly (two fissions).

7. The stability of the cytoplasmic states is a characteristic of each stock, and is presumably controlled by hereditary factors. The nature of these factors—whether they are the antigen-determining genes themselves, whether they are other, "modifying," genes, or whether they are some non-genic hereditary elements—is at present unknown. The result of this variation in stability of cytoplasmic states, however caused, is that certain antigen-determining genes are more readily expressed, under given conditions, than are their alleles in other stocks.

Much of the above theory conforms with that previously developed by SONNEBORN (1951 and earlier) for antigen determination in variety 4 of *P. aurelia*. The present work therefore affords no support for the early view (SONNEBORN 1945) that variety 1 made use of a genetical mechanism substantially different from that of variety 4, namely that differences in the "A" varieties (variety 1) were gene controlled only, while differences in the "B" varieties (variety 4) showed both genic and cytoplasmic control. We can now assert with confidence that both groups of varieties make use of essentially the same genetic system, involving both genes and cytoplasm. In variety 1, certain features of this system are now apparent which in variety 4 were less clear. They have been made clear as a result of the discovery that the antigen types formed at particular temperatures in one stock correspond both serologically and genetically with the antigen types formed at the same temperatures in other stocks. This has facilitated a more detailed genic analysis in variety 1, and led finally to the conclusion that cytoplasm which is in a state favorable for the expression of a particular gene, also favors the expression of alleles of that gene.

The earlier results of SONNEBORN (1943) and KIMBALL (1947) with variety 1 antigens can be readily accommodated in the theory adopted here. SONNEBORN obtained evidences of a single genic difference between stocks 1 and 60, from segregation of antigenic types derived from the two stocks. Similar segregations have many times been obtained in this work (see table 4). KIMBALL, using stock 60, by various treatments, including exposure to low temperature, was able to induce a transformation to "resistance" to a particular antiserum. It is now assumed that this transformation involved the substitution of a new antigen in place of the old (probably 60S in place of 60G), and not merely the loss of the old antigen, as thought by KIMBALL.

As regards the nature of the cytoplasmic materials responsible for production of the antigens, it must be admitted that we have at present very little information. It was formerly considered (SONNEBORN 1947b; SONNEBORN and BEALE 1949) that in variety 4 of *P. aurelia* the antigens were determined

by the activity of certain gene-like particles in the cytoplasm (plasmagenes). This was assumed because (1) antigenic variations within a stock were hereditary, persisting under certain conditions indefinitely, and (2) they were inherited through the cytoplasm. Subsequently, however, it was shown by SONNEBORN (1950b), that the specificity of some antigens was under strict gene control. For example, in variety 4, stocks 51 and 29 both produced a type designated A, though careful tests revealed a clear serological difference between the antigens 51A and 29A. Breeding tests showed that this difference was controlled by a single gene.

Furthermore, no evidence has yet been forthcoming to show the existence in the cytoplasm of any essential reproducible particles controlling the antigens. If a given antigen is replaced by another as a result of some change in the cytoplasm, the loss of the original antigen is never final; by changing the conditions it can always be recovered, provided only that the corresponding gene has not been replaced. There seem to be no elements in the cytoplasm which, once lost, cannot be recovered as a result of the activities of certain genes.

The new data presented here reinforce these doubts concerning the plasmagene type of hypothesis, in so far as the antigens are concerned. (The situation with regard to kappa is totally different, see SONNEBORN 1950b; BEALE 1951). Every antigen investigated in variety 1 has its specific controlling gene; variations in the state of the cytoplasm can be readily brought about, or reversed, by changing the temperature. We do not at present understand the cause of the variation in stability of the different cytoplasmic states, but that may also turn out to be genically controlled.

In order to prove the existence of autonomous cytoplasmic factors, it might be thought sufficient to demonstrate that two or more diverse kinds of cytoplasm could exist under identical genic and environmental conditions, that is, that variations in the cytoplasm could occur independently of variations in genes or environment. Such evidence was provided by the variety 4 antigen system, in which it was shown by SONNEBORN and LESUER (1948) that types A, B and D of stock 51 could be maintained indefinitely by growth at 26° with one fission per day. In variety 1 there are indications of similar phenomena. For example, animals of stock 90, grown at 29° with restricted food (sufficient for three fissions per day), remain for long periods either type 90G or 90D, whichever happens to be present at the beginning. Transformations from G to D and the reverse do however occur at rare intervals. Probably, under other conditions, complete stability of the two types could be obtained.

It has however been pointed out by DELBRÜCK (see SONNEBORN and BEALE 1949) that even in the variety 4 system, it is not necessary to postulate the existence of autonomous cytoplasmic factors, or as they are called, units "endowed with genetic continuity," in order to account for the observed behavior of the cytoplasm. It could be due to the operation of a flux equilibrium system capable of functioning in several ways under identical conditions.

We have to admit that there are at present no data which would permit us

to establish either the material nature of the cytoplasmic states, or what determines them. It is conceivable that they are determined by the activity of certain genes, whether connected with the antigen-determining system or not. On this view, a series of genes would liberate into the cytoplasm substances which would each correspond to a particular cytoplasmic state. There would be a competition between these substances, since only one cytoplasmic state is formed at any one moment, and the result of this competition would be decided, ultimately, by the external environment. Once established, a particular cytoplasmic state would persist at the expense of the other, competing, states through the operation of some such mechanisms as that postulated by DELBRÜCK. This is admittedly highly speculative and we must concede that a satisfactory solution of these problems awaits further experimental data. It is quite possible that some totally different explanation may turn out to be the correct one.

Whatever may be the true nature of the cytoplasmic elements responsible for these phenomena, their principal value lies in the model they provide for cellular differentiation in higher organisms, as SONNEBORN (1947b) has pointed out. Since most cells of a higher organism are presumed to contain sets of the same genes, differentiation is thought to occur through the development of diverse cytoplasms. The results of the experiments here described exemplify how such a mechanism might function. Certain cells in a developing organism are exposed to the action of a changing environment, which could bring about a change in the state of the cytoplasm. This in its turn would permit the expression of certain genes, and inhibit the expression of others, thus leading to the production of diverse cells. In the antigen system there is a limited number of sharply distinct cytoplasmic states (the number being limited by the number of gene-loci operating in the system) and this would parallel the occurrence, in cellular differentiation, of a number of sharply distinct types of cell. As WADDINGTON (1950) stresses, there is not an unlimited number of types of cell, with innumerable intermediates.

It has been possible to devise this model, not because the genetics of the *Paramecium aurelia* is in any fundamental way different from that of higher organisms, but simply because certain types of experiment are at present possible with Paramecium alone. This is due to two facts: (1) cells of Paramecium can be exposed to diverse environments, in such a way as to bring about long-lasting modifications, and (2) such modified cells can be caused to undergo conjugation, a process which permits a separation of nuclear from cytoplasmic factors.

SUMMARY

1. In *Paramecium aurelia,* variety 1, genes at three independent loci *g, d* and *s* have been shown to be concerned with antigen production. Each stock of paramecia contains characteristic alleles at each of these loci. So far, four alleles have been demonstrated at the *g* locus, three at the *d* locus and two at the *s* locus. The antigens corresponding to allelic genes usually show serological relationships.

2. Only genes at one of the three loci are normally expressed at a given time. Which one is expressed depends upon the state of the cytoplasm. There is a cytoplasmic state favorable for the expression of the *g* alleles, another cytoplasmic state favorable for the expression of the *d* alleles, and a third for the *s* alleles.

3. The state of the cytoplasm is determined to some extent by the temperature. At 29°–33°, the *d* alleles are normally expressed; at 25° the *g* alleles, and at 18° the *s* alleles. Modification of cytoplasmic state by change of temperature may be subject to prolonged delay, but the actual transformation from one state to another is a relatively sudden event.

4. The stability of corresponding cytoplasmic states under standard environmental conditions varies widely in different stocks.

5. The system of antigen-determination in variety 1 of *P. aurelia* does not differ in any essential respect from the variety 4 system previously described.

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