SEROLOGICAL INVESTIGATIONS OF CELL ADHESION IN THE SLIME MOLDS, DICTYOSTELIUM DISCOIDEUM, DICTYOSTELIUM PURPUREUM, AND POLYSPHONDYLIUM VIOLACEUM*

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According to the Tyler-Weiss hypothesis the cells of multicellular organisms are held together by forces similar to those of an antigen-antibody reaction (Tyler, 1947; Weiss, 1947). The interactions of dissociated sponge cells described by Wilson (1907, 1910, 1930, 1932) and Galtsoff (1923, 1925 *a*, *b*, 1929) and the work of Holtfreter (1944) on embryonic tissue cells have been cited by Tyler (1947) as examples in which such forces may be involved. Spiegel (1954 *a*, *b*) studied the effects of various types of antisera on dissociated sponge cells and embryonic amphibian tissue cells which provided additional evidence consistent with the Tyler-Weiss hypothesis.

The normal development of the slime molds, *Dictyostelium discoideum*, *Dictyostelium purpureum*, and *Polysphondylium violaceum* includes an aggregation process. This process involves the organization of large numbers of previously independent amebas into a unit which is capable of coordinated morphogenetic movements. While the assembling of the slime mold amebas in the initial phase of the aggregation process is evidently a chemotactic response (Bonner, 1947), it is possible that the specific union (adherence) of the cells involves an antigen-antibody type of reaction. Since the cells are intimately associated during morphogenesis their surfaces must play an important role in development. It seems mandatory that some mechanism exists in these cells such that apposing surfaces are caused to maintain contact but retain a degree of mobility.

The slime molds present a system in which the cells initially exist independently followed by a multicellular existence in which certain demands are imposed upon their surfaces. Under these circumstances the cell surfaces might undergo changes detectable by immunological methods and these changes may be necessary for cell adhesion.

Von Schuckmann (1925) demonstrated that the injection of *D. mucoroides* mature sorocarps into the rabbit produced an antiserum which was capable of

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agglutinating vegetative amebas of the same species. Zaczynski (1951) found that *D. discoideum* antiserum effected agglutination of homologous vegetative amebas. This phenomenon of agglutination was utilized in the present study of surface antigens during the morphogenesis of the slime molds, *D. discoideum*, *D. purpureum*, and *P. violaceum*.

Methods and Materials

Preparation of Antisera.—The slime molds, *D. discoideum*, *D. purpureum*, and *P. violaceum* were cultured according to Bonner's method (Bonner, 1947). The cultures were grown at 25°C. for 48 hours. Therefore, many of the amebas were undergoing aggregation or were on the verge of aggregation. The slime molds were washed from nutrient agar plates (four plates/species) with distilled H_2O and centrifuged

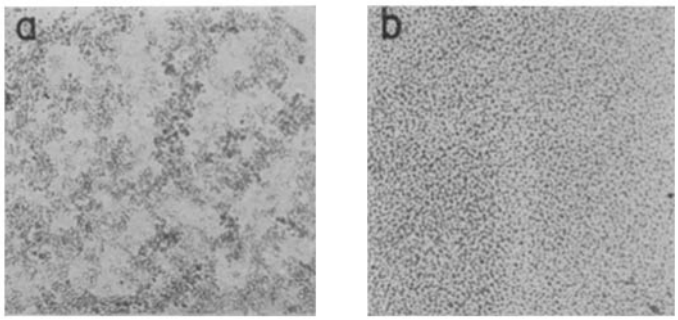

FIG. 1 *a*. Demonstrates the agglutination of *D. discoideum* amebas by anti-*D. discoideum* serum. × 140.

FIG. 1 *b*. *D. discoideum* amebas unagglutinated by preinjection serum (*D. discoideum* control). × 140.

gently to separate the amebas from the majority of the bacteria. Each time the amebas were prepared they were adjusted to a certain optical density with a Coleman colorimeter. This procedure made it possible to standardize the number of amebas for injection into rabbits. Samples of blood for preinjection sera (controls) were taken from the rabbits by heart puncture, using sterile procedure, before the immunizing antigen injections were begun. The blood was allowed to stand at room temperature for about 1 hour before being placed in a refrigerator overnight. The sera were then obtained by centrifugation and stored in serum bottles in the freezing compartment of a refrigerator. The sera were heated at 56°C. for 30 minutes in order to destroy complement.

A series of nine intravenous injections of the cell suspensions of the three species of slime molds were given over a period of 29 days to mature rabbits through the marginal vein of the ear. The nine injections involved a total of 27 ml./rabbit containing 0.21 mg. total N/ml. cell suspension. The initial injection was 1.0 ml. Each succeeding injection was increased by 0.5 ml. until the final injection of 5.0 ml. The rabbit injected with *P. violaceum* died following the ninth injection. The anti-*D.*

discoideum and anti-*D. purpureum* sera were obtained by heart puncture 13 days following the last injection and were prepared in the same way as previously described for the preinjection sera. All sera were stored in the freezing compartment of a refrigerator until ready for use.

Determining the Presence of Antibodies.—The test antigens were prepared by washing amebas of the three species of slime molds, *D. discoideum*, *D. purpureum*, and *P. violaceum* relatively free of bacteria (*E. coli*). The presence or absence of antibodies was determined by placing a drop of a concentrated suspension of amebas (approximately 1.5×10^8 cells/cc.) on a glass coverslip and adding a drop of antiserum of approximately equal volume. The preparation was stirred with a fine glass rod. As a result the cells were arranged in a film and were quite close together. In the event of a positive test the amebas agglutinated within seconds forming clumps resembling agglutinated red blood cells (Fig. 1 *a*, *b*). To detect any possible interference from *E. coli* antibodies the antisera were absorbed by *E. coli* before certain experiments. No consistent difference could be found between absorbed and unabsorbed antisera. Therefore, the effect of *E. coli* antibodies on the agglutination of slime mold amebas was assumed to be negligible.

RESULTS

Titers of Anti-D. discoideum and Anti-D. purpureum Sera.—The titers of the anti-*D. discoideum* and anti-*D. purpureum* sera were determined by diluting the antisera and noting the greatest dilution capable of effecting agglutination of homologous amebas (approximately 1.5×10^8 cells/cc.) by the end of a 10 minute period. By this method the anti-*D. discoideum* serum was determined to be 1/400 for a 27 hour culture of *D. discoideum* while that of the anti-*D. purpureum* serum proved to be 1/200 for a 28 hour culture of *D. Purpureum*.

Anti-D. discoideum Serum.—An examination of Table I will show that anti-*D. discoideum* serum is capable of agglutinating *D. discoideum* amebas from 24 to 48 hour cultures. However, anti-*D. discoideum* serum fails to agglutinate both *D. purpureum* and *P. violaceum* amebas from 24 to 26 hour cultures. Anti-*D. discoideum* serum will agglutinate both *D. purpureum* and *P. violaceum* amebas from 30 to 48 hour cultures.

No positive agglutinations of the three species of amebas from 24 to 36 hour cultures were observed in preinjection serum (*D. discoideum* control). Very slight agglutination of *D. purpureum* and *P. violaceum* from 43 to 48 hour cultures was sometimes observed in preinjection serum (*D. discoideum* control).

Anti-D. purpureum Serum.—Table I shows that anti-*D. purpureum* serum is capable of agglutinating *D. purpureum* amebas from 24 to 48 hour cultures. Anti-*D. purpureum* serum fails to agglutinate both *D. discoideum* and *P. violaceum* amebas from 24 to 26 hour cultures. However, anti-*D. purpureum* serum effects the agglutination of both *D. discoideum* and *P. violaceum* amebas from 30 to 48 hour cultures.

Positive agglutinations were not observed in any of the three species of

amebas from 24 to 36 hour cultures in preinjection serum (*D. purpureum* control). Very slight agglutinations of *D. purpureum* and *P. violaceum* amebas from 43 to 48 hour cultures were observed occasionally in preinjection serum. In one trial *D. purpureum* amebas from a 72 hour culture were observed to agglutinate in preinjection serum (*D. purpureum* control).

TABLE I

The Activity of Anti-D. discoideum, Anti-D. purpureum, and Corresponding Preinjection Sera (D. discoideum and D. purpureum Controls) on D. discoideum, D. purpureum, and P. violaceum Amebas

All sera were diluted to one-half as a result of the addition of amebas. + = positive agglutination, − = no agglutination, and ± = very slight agglutination. The numbers preceding the symbols refer to the number of separate trials.

Type of serum	Age of cultures	Test antigens								
		D. discoideum			*D. purpureum*			*P. violaceum*		
	hrs.									
Anti-*D. discoideum* serum	24–26	9+				9−			2−	
	30–36	12+			7+	2−	2±		2−	4±
	43–48	12+			9+	2−	1±	5+	2−	1±
Preinjection serum (*D. discoideum* control)	24–26		3−			3−				
	30–36		4−			4−			3−	
	43–48		7−			3−	3±		3−	1±
Anti-*D. purpureum* serum	24–26		7−		8+				2−	
	30–36	8+	4−		9+				2−	4±
	43–48	5+	4−	1±	11+*		2±	4+	2−	1±
Preinjection serum (*D. purpureum* control)	24–26		2−			2−				
	30–36		5−			4−			3−	
	43–48		7−		1+*	3−	4±		3−	1±

* Includes one agglutination from a 72 hour culture.

DISCUSSION

By virtue of their particular developmental pattern the slime molds present an unusual opportunity to study the role of cell surfaces during development. The vegetative amebas at first exist as independent units but assume the status of a multicellular organism upon aggregation. Therefore, any detectable surface changes which occur during the aggregation period when the cells make contact might be described as changes necessary to some degree for maintaining the integrity of the aggregate.

The present data may be interpreted in the following manner: It may be assumed that certain surface antigens of 24 to 26 hour *D. discoideum*, *D.*

purpureum, and *P. violaceum* amebas are species-specific and persist through later stages of development. Such antigens may be assigned the symbols D, P, and V, respectively. The anti-*D. discoideum* and anti-*D. purpureum* sera, produced by injecting 48 hour amebas into rabbits, contain then antibodies which are species-specific and which may be assigned the symbols anti-D and

ANTIBODIES		ANTIGENIC STRUCTURE OF THE SLIME MOLD SURFACES					
		D. DISCOIDEUM		D. PURPUREUM		P. VIOLACEUM	
		24 TO 26 HOUR	30 TO 48 HOUR	24 TO 26 HOUR	30 TO 48 HOUR	24 TO 26 HOUR	30 TO 48 HOUR
		D D	D N D N	P P	P N P N	V V	V N V N
D. DISCOIDEUM	ANTI-D	+	+	−	−	−	−
	ANTI-N	−	+	−	+	−	+
	ANTI-D + ANTI-N	+	+	−	+	−	+
D. PURPUREUM	ANTI-P	−	−	+	+	−	−
	ANTI-N	−	+	−	+	−	+
	ANTI-P + ANTI-N	−	+	+	+	−	+

FIG. 2. Hypothetical scheme of identical and complementary antigenic structures of two apposing surfaces of *D. discoideum*, *D. purpureum*, and *P. violaceum* amebas, of the types of antibodies produced by 48 hour *D. discoideum* and *D. purpureum* amebas and the pattern of agglutination they effect consistent with the present data. + = agglutination, − = no agglutination.

anti-P respectively. Agglutination, therefore, would occur in those instances in which amebas of any age and homologous antisera are interacted. Hence anti-D + D or anti-P + P results in agglutination. Fig. 2 illustrates the types of antibodies which might be produced by using 48 hour amebas as the immunizing antigen. This scheme of antibody production is discussed by Tyler (1955).

In addition to species-specific surface antigens, D, P, and V, the 30 to 48 hour cultures of *D. discoideum*, *D. purpureum*, and *P. violaceum* contain surface

antigens which are non–species-specific and which arise only during this later stage. These antigens will be termed N. The two antisera, in addition to anti-D and anti-P antibodies, also contain anti-N. Therefore, agglutination of *D. discoideum*, *D. purpureum*, and *P. violaceum* amebas of the 30 to 48 hour stage by heterologous antisera would be effected by the interaction of anti-N + N (Fig. 2).

When antisera prepared by injecting amebas from 48 hour cultures of *D. discoideum* and *D. purpureum* into separate rabbits are mixed with *D. discoideum*, *D. purpureum*, and *P. violaceum* amebas from 24 to 26 hour cultures the anti–*D. discoideum* and anti–*D. purpureum* sera fail to agglutinate the heterologous amebas. However, if the anti–*D. discoideum* and anti–*D. purpureum* sera are mixed with amebas of the three species from 30 to 48 hour cultures, either antiserum will effect agglutination of all three species.

The data suggest that perhaps the surface antigens in the vegetative amebas (24 to 26 hour cultures) are not concerned with cell contacts or adhesion. However, as the time of aggregation is approached, it appears that additional surface antigens arise which are common to all three species. These may well be concerned with cell adhesion and the integration of morphogenetic movements in some as yet not understandable manner (Fig. 2). The antigenic structures revealed serologically, however, are not necessarily identical with those involved in maintaining cell adhesion. Occasionally, spontaneous agglutinations (not to be confused with the normal process of aggregation which occurs slowly) of *D. purpureum* amebas and also *P. violaceum* amebas from 43 to 48 hour cultures occurred in preinjection sera. This appears to lend support to the idea that new antigens concerned with cell adhesion arise during the aggregation process, especially since the vegetative amebas of these two species from 24 to 26 hour cultures did not show spontaneous agglutination. The presence of antigens concerned with cell adhesion might be disadvantageous to amebas during the period of active feeding, cell division, and growth.

Developmental manifestations of surface antigen changes may well have been demonstrated by the work of Raper and Thom (1941). When vegetative amebas of *D. discoideum* and *D. purpureum* were combined the resulting fruiting bodies were characteristic of their particular species. However, when migrating pseudoplasmodia of *D. discoideum* and *D. purpureum* were thoroughly mixed together, a certain proportion of the fruiting bodies were composed of both species of amebas. A parallel may be observed here in that morphogenetic interaction between the cells of *D. discoideum* and *D. purpureum* does not occur until the appearance of similar surface antigens (Table I). It appears, therefore, that the similarities in the surface antigens which arise during the developmental stages are of sufficient magnitude to oppose any tendency of the amebas of either species to sort out in every instance. Raper and Thom (1941) have also shown that mixtures of *D. discoideum* and *P.*

violaceum fail to form mosaic fruiting bodies regardless of the stage at which they were combined. Although anti-*D. discoideum* serum is capable of agglutinating 43 to 48 hour *P. violaceum* amebas it is suggested that the similarities in surface antigens are not of sufficient magnitude to effect morphogenetic interaction between the cells of these two genera. There is no quantitative evidence available, however, which demonstrates the relative similarity of the surface antigens of these three species of slime molds. There are undoubtedly genetic and physiological differences preventing *D. discoideum* and *P. violaceum* amebas from undergoing development when combined and the lack of a surface configuration of sufficient similarity is suggested as one of these factors.

Therefore, it appears that surface antigens of the amebas form new antigens (while still retaining some of the earlier antigens) as the time of aggregation is approached. Furthermore, these data suggest that cells in order to undergo coordinated morphogenetic movements must be associated with cells bearing a sufficient number of similar surface antigens. Cells with relatively dissimilar surfaces would be apt to reject each other or undergo uncoordinated movements. According to the interpretation presented here, the surfaces of the cells of any one species undergoing aggregation are assumed to be identical and to bear antigenic structures that are complementary as well as identical (Fig. 2). The data in this study support the Tyler-Weiss hypothesis and the views on functional manifestations of cell adhesion expressed by Tyler (1955).

SUMMARY

The author is indebted to Dr. Janet Twente and Miss Ruth Bronsweig for research assistance during the course of this study. Furthermore, appreciation is extended to the Department of Pharmacology for the many facilities made available to us. Acknowledgements are also due Professor Albert Tyler of the California Institute of Technology for critically reading this manuscript.

1. Antibodies to slime molds were produced by injecting *D. discoideum* and *D. purpureum* amebas from 48 hour cultures into rabbits.

2. Anti-*D. discoideum* and anti-*D. purpureum* sera caused agglutination of homologous amebas from 24 to 26 hour cultures, agglutination of certain heterologous amebas from 30 to 36 hour cultures, and agglutination of all heterologous amebas from 43 to 48 hour cultures.

3. The data show that new surface antigens are formed in cultures after 26 hours and it is suggested that the new antigens are concerned with cell adhesion.

4. The probable role of surface antigens in the interaction of cells of different species of slime molds was discussed.

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