THE INDUCTION OF MUTATIONS BY ANTIBODIES*

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In most biological syntheses each step is controlled by a different gene. This conclusion was suggested by such combined genetic and biochemical studies as those on the anthocyanin pigments[1] from which it appeared that a particular gene regulates the oxidation (or methylation, or the nature of the glucosides) at particular carbon atoms of the anthocyanin molecules. Studies of the eye pigments of Drosophila[2] and the hair pigments of guinea pigs[3] each indicate that the different steps in a particular synthesis are controlled by different genes. Almost overwhelming support for the conclusion is coming from studies of "biochemical" mutations in Neurospora.[4]

Studies of inherited antigenic differences in many species[5] agree in showing that the immunological specificity of an individual antigen is determined by a single gene, as if the development of antigenic specificity represents but a single step in the synthesis of the antigen. The synthesis of genes may represent an analogous instance. "The most fundamental —in fact, the unique and distinctive—characteristic of a gene is . . . the fact that, in its protoplasmic setting, it produces a copy of itself, next to itself, and that when its own pattern becomes changed, the copy it now builds is true to its new self."[6] In the terminology of this report: the specificity of a gene is determined solely by a gene of identical specificity to that synthesized.

The possibility that a gene impresses a copy of its own antigenic specificity on the antigen it regulates can, under sufficiently favorable circumstances, be tested experimentally. Antibodies to such cellular antigens should combine with the genes whose antigenic structure is the same as that of the immunizing antigen. There is no certainty that the gene-antibody reaction can be recognized by the usual serological techniques. There is the chance, however, that if antibodies combine with genes at the time they are "producing copies of themselves," the presence of the antibody molecule would so alter the surface of the gene that the copy produced would have a changed structure. Such alteration in genic structure should, on the basis of this reasoning, result in some change in the physiological or developmental step regulated by the gene in question, and the antibody-induced change should be recognizable as a mutation, as pointed out by Sturtevant in the preceding paper.[7]

While the series of events just postulated presents numerous conditions to be met in an experimental attack, there are certain recorded cases which

appear to fit this interpretation. Of these the only one in which antibodies to a relatively pure antigen have induced changes of proved heritable nature is that reported by Guyer and Smith and discussed by Sturtevant.[7] The instances of apparent induction of mutations in bacteria by specific antisera, while suggesting the same interpretation, do not fulfil all conditions since the absence of sexual reproduction in these organisms makes the genetic proof of mutation impossible. Probably the most thoroughly worked out example of changes following treatment with antisera is the smooth to rough transformation in pneumococcus.[8] The smooth, or virulent, form produces a polysaccharide envelope (chemically and immunologically specific to the type) which is absent in the rough form. Changes from smooth to rough rarely occur spontaneously, but are abundantly produced by growth in antiserum to the specific type. While, from the published accounts, it seems probable that the loss of the capacity to synthesize the polysaccharide is directly induced by antibodies to the polysaccharide, it is still impossible to rule out the alternative interpretation that the antibodies act as a powerful selective agent favoring those rough cells which occasionally arise spontaneously.

The ascomycete, *Neurospora crassa*, was selected for the experiments to be reported because of its favorable genetic and cultural characteristics.[9] The general plan of the experiments was to treat the fungus with antisera (developed in rabbits to mycelial extracts or culture filtrates), isolate strains descended from single nuclei (obtained by crossing to the opposite sex and selecting ascospores), and to test the isolates for deficiencies in growth on different carbohydrates.[10] The results of the experiments are summarized in the accompanying table.

To test the genetic homogeneity of the strains used (Beadle's 1A and 25a) conidia of one sex were dusted over cultures of the other sex in which properithecia had already developed. Since the nuclei from one conidium do not ordinarily take part in fertilization in more than one perithecium,[11] it is possible to sample genes from a large number of nuclei of the conidial parent by testing one isolate from each perithecium. The untreated controls listed in the table were handled in this way. Whenever conidia were treated with antisera the isolates were obtained in the same way, but in those instances in which the mycelial stage was treated the method was modified, and there is the possibility that more than one isolate was descended from a single treated nucleus.

The data indicate a much greater variability in the treated material than in untreated controls. No statistical analysis is attempted because the tests for variants were not strictly comparable from one experiment to another. In any one experiment, however, the controls were tested at the same time and in the same way as the treated isolates, and the differences are consistent throughout.

Of the variants listed, eleven have so far been subjected to genetic test, and each of these has differed from the wild-type parents by a single gene. Evidence from genetic tests and from tests of heterocaryons[12] indicates that four variants (Nos. 1077, 1083, 1092 and 1095) from one treatment represent different alleles of a gene by which the parent strains (1A and 25a) differ from another pair of wild-type strains (Beadle's 4A and 12a). At least three other variants represent mutations at three other loci.

MATERIAL TREATED	TREATMENT	IMMUNIZING ANTIGEN	CROSSED TO	TOTAL ISOLATES	VARIANTS NO.	VARIANTS IDENTIFICATION
Mycelium of 25a	50% antiserum, 27 hrs. at 25°	Mycelial extract	Untreated 1A	16	1	No. 859
	50% antiserum, 27 hrs. at 25°	Culture filtrate	Untreated 1A	21	0	...
	50% normal serum, 27 hrs. at 25°	None	Untreated 1A	12	0	(Control)
Conidia of 1A	100% antiserum, 3 hrs. at 25°	Culture filtrate	Untreated 25a	52	4	Nos. 1077, 1083, 1092 1095
	None	None	Untreated 25a	63	0	(Control)
Mycelium of 1A	100% antiserum, 48 hrs. at 25°	Culture filtrate	25a, same treatment as 1A	128	1	No. 1874
	Same, but two 2-day transfers	Culture filtrate	25a, same treatment as 1A	71	2	Nos. 1905, 2606
	Same, but four 2-day transfers	Culture filtrate	25a, same treatment as 1A	210	2	Nos. 2195, 2679
	Same, but five 2-day transfers	Culture filtrate	Untreated 25a	94	1	No. 2012
Conidia of 1A	None	None	Untreated 25a	170	0	(Control)
Conidia of 25a	100% antiserum overnight at 2°	Starch adsorbed enzymes	Untreated 1A	13	2	Nos. 5131, 5168
	100% antiserum overnight at 2°	Starch adsorbed enzymes	Untreated 1A	30	4	Nos. 5175, 5177, 5269, 5294
	100% antiserum overnight at 2°	Amm. sulphate preciptated culture filt.	Untreated 1A	31	3	Nos. 5139 5191, 5201
	100% antiserum overnight at 2°	Amm. sulphate precipitated culture filt.	Untreated 1A	29	5	Nos. 5007, 5008, 5031, 5204, 5211
	None	None	Untreated 1A	31	0	(Control)
			Total treated	695	25	
			Controls	276	0	

With the exception of two morphological mutations, all variants so far obtained differ from the wild type by deficient growth [13] when cultured at standard temperatures and on carbohydrate substrates normally utilized by the wild type. None has yet been obtained which fails to use a particular carbon source under all conditions.

The data just presented indicate that antibodies (or possibly some other serum constituent whose rôle is unsuspected) influence the fre-

quency of recoverable mutations. The indications are that the mutations are induced rather than selected by the treatment, since prolonged treatment of mycelium (during which considerable growth occurs) seems to be less effective than a relatively short treatment of conidia (where few nuclear divisions can have occurred). This difference was especially noticeable when conidia were treated at low temperatures which tend to favor more complete antigen-antibody reactions (e.g., in the precipitian and complement-fixation tests). The apparent deficiency of variants following prolonged growth in antiserum can be interpreted either as a result of selection against the mutated nuclei, or on the assumption that nuclei already affected are more likely to be further affected by antibodies, resulting in inviability.

While the immunizing solutions were known to contain many carbohydrases, the antibodies produced have not been identified in terms of specific antigens.[14] On the present interpretation, the four members of an allelic series may have arisen in response to one sort of antibody, the three independent genes to three other sorts.

The material seems well suited to the problem under discussion, but there are certain refinements to be adopted. Fertile wild-type stocks which lack the growth deficiencies of 1A and 25a, and which give as little variation as possible when intercrossed are being developed. Further, in order to observe a direct relationship between specific genes and antibodies it will be necessary to make use of antigens which can be isolated in relatively pure form. It may be essential to select antigens which are not in themselves indispensable to the organism, otherwise viable mutations are not to be expected.

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[1] References in Scott-Moncrieff, R., *Jour. Genetics*, **32**, 117–170 (1936), and Lawrence, W. J. C., and Price, J. R., *Biol. Rev.*, **15**, 35–58 (1940).

[2] References in Beadle, G. W., and Tatum, E. L., *Amer. Nat.*, **75**, 107–116 (1941), and Ephrussi, B., *Quart. Rev. Biol.*, **17**, 327–338 (1942).

[3] References in Wright, S., *Biol. Symposia*, **4**, 337–355 (1942).

[4] Beadle, G. W., and Tatum, E. L., *Proc. Nat. Acad. Sci.*, **27**, 499–506 (1941); Tatum, E. L., and Beadle, G. W., *Growth, Fourth Growth Symposium*, **6**, 27–35 (1942), etc.

[5] For example, the heritable erythrocyte antigens of man (Landsteiner and others), of fowls (Todd), of rabbits (Castle and Keeler), see references in Wiener, S., *Blood Groups and Blood Tranfusion*, 2nd ed., Charles C Thomas, Springfield, Ill., 1939; references to the Rh-factor in man in Wiener, A. S., and Landsteiner, K., *Proc. Soc. Exp. Biol. Med.*, **53**, 167–170 (1943); erythrocyte antigens of doves, Irwin, M. R., *Genetics*, **24**, 707–721 (1939); of cattle, Ferguson, L. C., Stormont, C., and Irwin, M. R., *Jour. Immunol.*, **44**, 147–164 (1942); the serum protein antigens of doves, Irwin, M. R., and Cumley, R. W., *Genetics*, **27**, 228–237 (1942).

[6] Quoted from Muller, H. J., *Cold Spring Harbor Symposia on Quantitative Biology*, **9**, 290–308 (1941).

[7] Sturtevant, A. H., the preceding paper in these PROCEEDINGS.

[8] Reimann, H. A., *Jour. Exp. Med.*, **41**, 587–600 (1925), has references to earlier literature; Felton, L. D., *Ibid.*, **56**, 13–25 (1932); Paul, J. R., *Jour. Bact.*, **28**, 46–66 (1934). See also discussions in Heidelberger, M., *Amer. Nat.*, **77**, 193–198 (1943), and Avery, O. T., McLeod, C. M., and McCarty, M., *Jour. Exp. Med.*, **79**, 137–158 (1943). A different interpretation of the transformations in pneumococcus is given by Sonneborn, T. M., *Proc. Nat. Acad. Sci.*, **29**, 338–343 (1943).

[9] References in Lindegren, C. C., *Iowa State Col. Jour. Sci.*, **16**, 271–290 (1942), and Beadle and Tatum (1941), loc. cit., footnote 4.

[10] Tests were usually made on sucrose, cellobiose, maltose, starch and α-amylodextrin, on which the wild type grew "normally." The method is essentially similar to that described by Beadle and Tatum (1941), loc. cit., footnote 4.

[11] It has been shown by Backus, M. P., *Bull. Torrey Bot. Club*, **66**, 63–76 (1939), that conidia of *Neurospora sitophila* do not germinate on agar already supporting mycelial growth, but trichogynes from the properithecia attach themselves to such conidia and withdraw the cellular contents.

[12] The method of testing for allelism with heterocaryons is described by Beadle, G. W., and Coonradt, V. L., *Genetics*, **29**, 291–308 (1944).

[13] Growth characteristics were determined by the "tube method" described by Ryan, F. J., Beadle, G. W., and Tatum, E. L., *Amer. Jour. Bot.*, **30**, 784–799 (1943).

[14] Precipitin titres were too low to permit the utilization of an absorption technique.

A RECONSIDERATION OF THE MECHANISM OF POSITION EFFECT

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The phenomenon of position effect has come in recent years to occupy a central position among problems facing the geneticist, in spite of the restriction of well-established cases to one species. This is, no doubt, due partly to the fact that position effect now clearly appears to be much more frequent in Drosophila than originally suspected; but even more to its possible bearing on the problem of the degree of discreteness of the hereditary material. The real significance of position effect in this connection will depend, of course, on the nature of the mechanism which will be found to be responsible for its occurrence; and that is why the clarification of this mechanism appears so urgent.

Speculations concerning the mechanism of position effect have, on the whole, been surprisingly timid. With the exception of the two papers by Muller,[1] and Offermann,[2] especially devoted to the theoretical discussion of this problem and of Schultz's papers[3, 4] on variegation, the literature on