space charge limits the positive ions to the end-plates to the current value of little more than one half the positive ion current from the main arc.

The cylinder being insulated receives an equal number of positive ions and electrons per unit time. Hence, $\bar{V}_{1r} - \bar{V}_{2r} = 0$ there. The insulated cylinder has also nearly zero resistance. Therefore, $\bar{V}_{10} - \bar{V}_{20} = 0$ and $V_{1z} - V_{2z} = 0$.

On the other hand, from F&P(7.29) we have $\rho_1\bar{V}_1 + \rho_2\bar{V}_2 = 0$. Hence, we have six scalar homogeneous equations satisfied by all six components of $\bar{V}_1$ and $\bar{V}_2$ at the cylinder, and we conclude they are all zero there. Hence, the beam of particles to the cylinder must consist of both positive ions and electrons mixed together with like number densities and like velocities. From the fact that the end-plates receive half of the positive ions from the arc and practically no electrons, we get that the velocity of the ion is at 45° to the cylinder surface, and its total energy per ion is $(m_1 + m_2)v_0^2/2 = (e/8\ \pi c^2 m_1)\mathbf{B}^2r^2$ and the energy per electron is very small, that is zero.

Now for the second type of discharge of my paper,[2] pp. 315–317. If we assume that the whole electron current to the end-plates is limited to a small value by space charge, then the whole ion current is carried (with electrons) at low voltage to the cylinder. But in passing from the main arc where its energy is low across the magnetic field, the energy of the ions must be very high if they are not to be caught by the end-plates, and then low again at the cylinder where they are caught at low voltage.

The application to nuclear fusion power of this type of discharge is described in the last paragraph of my paper.[2] It has not yet been tried out.

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*THE STRUCTURAL BASIS FOR GENETIC VARIATIONS OF NORMAL HUMAN γ-GLOBULINS**

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Genetic differences (allotypes) in γ-globulins have been noted in rabbits,[1, 2] mice,[3] guinea pigs,[4] and humans.[5–11] Since the initial observation by Grubb[5] that human γ-globulins differ in their ability to inhibit the agglutination, by selected sera containing rheumatoid factor, of Rho (+) cells coated with incomplete anti D antibody, seven genetically determined variants in human γ-globulins have been described.[5–11] In Caucasians, two loci segregating independently control the production of 6 well defined factors. Genes at the Gm 1 locus determine the production of two main factors known as Gm 1 (*a*) and (*b*) which are inherited as simple codominant non-sex-linked alleles,[5, 6] and two minor ones known as Gm 1 (x)[7] and

(r).[8] The second locus (In V or Gm 2) also determines the production of two codominant allelic factors known as In V (*a*)[9] and (*b*).[10] The seventh factor, "Gm like" is absent from Caucasian subjects.[11] It is not linked to the In V factors and its relationship to the Gm 1 factors has not yet been clarified.[11] These γ-globulins can be distinguished from each other only by differences in their capacity to inhibit the agglutination of cells by selected sera containing substances serologically similar to, if not identical to, "rheumatoid factors," and to date no other characteristic chemical or structural differences have been found between them.

Studies of 3.5S units prepared from human 7S γ-globulin with papain and cysteine[12, 13] have shown them to consist of two fragments of slow electrophoretic mobility (A and C), each containing a single antibody combining site, and a third, more rapidly migrating fragment (B) which is devoid of antibody combining activity. The latter differs from the other two also in its antigenic properties[12] and the type of carbohydrate associated with it.[14] The determinant group(s) responsible for the cross-reaction of 7S γ-globulin with the other two immune globulins (19S γ and γ_{1A} globulins), as well as with the closely related pathologic myeloma proteins, macroglobulins, and Bence Jones proteins, are associated with the part of the molecule containing the antibody combining site, while the antigenic specificity of 7S γ-globulin resides primarily in fragment B.[15, 16]

The existence of two nonlinked genetic factors, together with the finding of two major chemically and immunologically distinct subunits of human 7S γ-globulin suggested the possibility of a separate genetic control of each subunit. The results of of the present studies present support for this concept in that Gm factors controlled by the two Gm loci reside in separate parts of the molecule; Gm 1 activity has been found only in fragment B, while In V activity was associated only with fragments A and C. In addition, In V activity, but not Gm 1 activity, has been found also in the 19S γ-globulins, γ_{1A} globulins, and certain Bence Jones proteins.

Materials and Methods.—Sera: Twenty to thirty ml of fresh normal serum were obtained on one or more occasions from 13 normal subjects chosen from a larger number on the basis of their Gm types. As shown in Table 1, they include each of the possible combinations of the 4 major groups at the Gm 1 and In V loci. Only the (*a*) and (*b*) factors at each locus were examined and Gm (x), (r), and "Gm like" factors were not analyzed further in this study. Six of the sera were examined on two or three separate occasions, and in each instance γ-globulin was freshly prepared from a new aliquot of serum and separately digested and analyzed.

Isolation of γ-globulins and papain fragments from 7S γ-globulin: 7S γ-globulin was isolated in the majority of experiments by batch chromatography on DEAE cellulose.[17] The preparations so obtained contained only 7S γ-globulins and were free of 19S γ and γ_{1A} globulins on immunoelectrophoresis. In three experiments, starch zone electrophoresis was employed for isolating the γ-globulins.[18] 3.5S fragments were prepared from 7S γ-globulin by digestion with papain, cysteine, and EDTA[12, 19] at 37°C for 15 hr. The reaction was stopped by dialyzing against phosphate buffer pH 8, μ 0.01 at 4°C. The two univalent fragments (A & C) were separated from fragment B by chromatography on DEAE cellulose.[12] Under the conditions employed, fragments A & C did not attach to the exchanger and came off the column together with the starting buffer (phosphate, pH 8, μ 0.01), while fragment B remained on the column and was subsequently eluted with the same buffer containing 0.3 *M* NaCl. In most of the experiments, A and C were not separated from each other because structurally as well as functionally they appear to be closely related to each other and may even represent similar fragments derived from γ-globulin molecules of different mobility.[12, 20, 21] In four instances, Fractions A and C were separated from each other by chromatography on carboxymethyl cellulose.[12] In each case, as illustrated with fractions from two sera in Table 2, they gave similar results in the Gm 1 (*a*) and (*b*) and In V (*a*) and (*b*) systems. Purity of the fragments was checked in each experiment by immunoelectro-

phoresis and Ouchterlony analysis, as described previously.[12] In no instance were fractions employed which contained more than an estimated 10 per cent contamination. In the early experiments, completeness of digestion was checked further by analytical ultracentrifugation. Subsequently, it was noted that undigested γ-globulin could be detected also by immunoelectrophoresis. In none of the preparations employed were traces of native 7S γ-globulin detected. γ_{1A}-globulins were isolated from pooled normal plasma by the procedure of Heremans[22] and 19S γ-globulins by repeated preparative ultracentrifugation as discussed in detail previously.[23] Neither preparation contained more than 5 per cent contaminating proteins.

Bence Jones proteins from the urines of 8 patients with multiple myeloma and one subject with Macroglobulinemia of Waldenström were studied initially. In 8 instances, sera were also available. Four of these had a γ myeloma spike, two had normal patterns, one was hypogammaglobulinemic, and one had a macroglobulin in the slow α_2 globulin region. The Bence Jones proteins were isolated by precipitation with 40–50 per cent saturated ammonium sulfate. They were redissolved in water and freed of salt by dialysis. Eight of the proteins gave only a single line on immunoelectrophoresis, which migrated with the γ or β globulins and, in one instance, with the α_2 globulins (ME). One (Bu) contained a second precipitin line. Each protein could be placed into one of two distinct antigenic groups by the Ouchterlony technique.[24] As demonstrated previously[24] and shown in Figure 1, these two classes of myeloma proteins differ from each other in antigenic structure, but each cross-reacts with fragments A and C and not with fragment B of 7S γ-globulin.[15] Thirteen additional Bence Jones proteins kindly supplied by Dr. E. Osserman were examined to confirm the results obtained with these proteins. In the latter instances, serum samples were not available for comparison.

Typing method and reagents: Two per cent washed 0 Rho positive red cells suspended in pH 7.2 phosphate buffered saline were sensitized at 37°C for one hr with an equal volume of anti Rho serum containing 160 "units" of antibody activity. (One unit is defined as the minimal amount of the anti Rho antiserum detected by the indirect Coombs' test). The sensitized cells were washed three times with saline and reconstituted to a 1.5 per cent suspension in phosphate buffered saline pH 7.2.

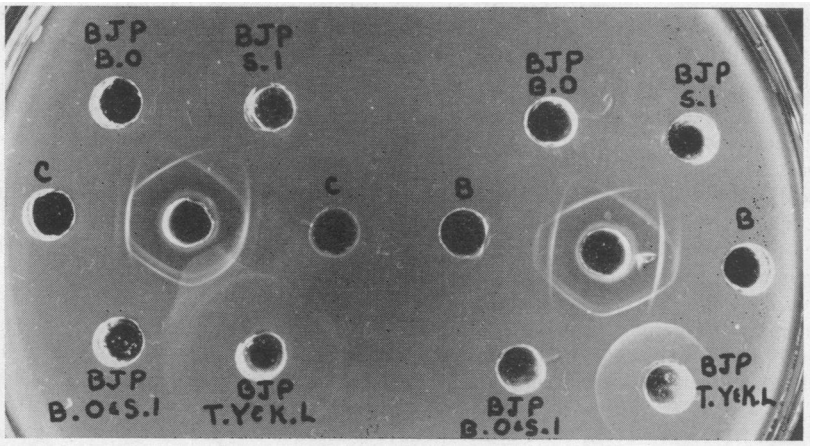

FIG. 1.—Comparison of the antigenic properties of Bence Jones proteins of types A & B and mixtures of these with fragments B or C of human 7S γ-globulin. Antiserum to 7S γ-globulin in the center wells.

One volume of serial dilutions (made with separate pipettes to prevent carry-over) of sera or fractions to be tested for inhibitory activity were added to an equal volume of a standard dilution of a rheumatoid serum of known specificity for one or another of the Gm or In V factors. The mixture was incubated in a 10 × 75 mm test tube at room temperature for 30 min. Then one volume of the sensitized cell suspension was added. After a 3-hr period of incubation at room temperature, the tubes were centrifuged for 45 sec in a serological centrifuge (Serofuge) and read for agglutination with a hand lens. Agglutination was graded from 0 to 3+ depending on the

degree of clumping. In all studies, appropriate controls were used to insure that agglutination was not due to the test serum or the added fractions rather than the "rheumatoid factor."

The test sera and red cell coating sera employed were similar to those used previously.[25] Each fraction was tested for inhibitory activity with at least two separate sets of reagents for each Gm factor with the exception of In V(*b*), for which only one set of reagents exists to date. In some of the later experiments, tests for In V (*b*) activity were not possible since the reagent was no longer available.

Inhibitory activity (+) or lack of inhibition (−) varies with the quality of the reagents used for the various test systems. For Gm 1 (*a*), the difference is striking and varies by a factor of at least 32 with both sets of reagents used. For In V (*a*), the difference is 8- and 16-fold with the two reagents used. For In V (*b*), the difference is 8-fold, and in the Gm 1 (*b*) system the difference between a positive and a negative serum is 4- to 8-fold with two sets of test reagents.

Results.—Recovery of Gm activity with whole 7S γ-globulins isolated by starch zone electrophoresis or chromatography was good in all instances. Gm 1 (*a*) activity was obtained at a concentration of 0.06 mg/ml; Gm 1 (*b*) was active at a concentration of 0.25–0.50 mg/ml, while In V (*a*) and (*b*) inhibited agglutination of the appropriate cells at concentrations of 0.125 mg/ml and 0.25 mg/ml respectively. In no instance was γ-globulin from a negative serum able to inhibit agglutination by the appropriate test serum following isolation. Results with papain fragments of 7S γ-globulin obtained from sera of varying combinations of Gm 1 and In V activity are summarized in Table 1. Following papain digestion and chromatographic separation of the fragments, Gm 1 (*a*) activity, i.e. the ability to inhibit agglutination of appropriately sensitized cells by an anti Gm (*a*) serum, was recovered only in fragment B, and in most experiments the inhibitory capacity per mg of protein was equal to or as much as two times greater on a weight basis than that of the native 7S γ-globulin from the same subject prior to digestion. No inhibition greater than expected on the basis of incomplete separation was ever noted in fragments A and C even when tested in concentrations 10 to 100 times greater than the minimal amount necessary for inhibition by the active fragment B. In the earlier experiments using small amounts, Gm 1 (*b*) activity could not be recovered in any of the fragments. When larger amounts of the fractions were used, inhibition was also observed only with fragment B. However, with one exception a concentration of 0.5–1.0 mg/ml of protein, or approximately twice the concentration of the native 7S protein, was required to inhibit. As was the case in Gm 1 (*a*) activity, inhibition was never associated with fragment A and C in concentrations as high as 4.0 mg/ml, nor was it recovered from Gm 1 (*b*) negative sera. While recovery of activity was poor and inconstant, the results suggest that it was present only in fragment B. The reason for the poorer recovery is not certain, but may be in part due to destruction of Gm activity by papain.[26]

In contrast to the results obtained with Gm 1 activity, In V activity (*a*) as well as (*b*) was not associated with fragment B but always found on fragments A and C. Here too, activity recovered in fragment B could be explained by small amounts of contaminating proteins. In general, the fragments were less active than native 7S γ-globulin, and in two instances In V activity was found in fragments prepared from a serum initially devoid of In V inhibitory activity when tested at high concentration. The reason for these discrepancies and for the somewhat lower inhibitory efficiency of the fragments compared to the native protein remains to be explained. As noted above, it may be in part due to changes during digestion and the fractionation procedures.[26] Detailed results of one titration with two sera,

TABLE 1

INHIBITORY CAPACITY OF FRAGMENTS A & C AND B FROM 7S γ-GLOBULIN FROM NORMAL SUBJECTS ON GM 1 AND IN V AGGLUTINATION SYSTEMS

	Gm 1 Locus						In V Locus					
	a			b			a			b		
Subject	Serum	A & C	B	Serum	A & C	B	Serum	A & C	B	Serum	A & C	B
S.W. 1*	+	Neg.	<0.05	−	(Neg.)	(Neg.)	+			+		
2		0.125†	0.002					0.12	Neg.			
B.F. 1*	−	(Neg.)	(Neg.)	+	Neg.	0.5	+	<0.1	Neg.	+		
2		(Neg.)	(Neg.)		Neg.	0.25		<0.1	Neg.		<0.1	Neg.
H.F. 1*	+	Neg.	0.05	+	Neg.	0.5	−	(Neg.)	(Neg.)	+		
2		Neg.	0.03		Neg.	1.0		(Neg.)	(Neg.)		<0.1	Neg.
D.A. 1	−	(Neg.)	(Neg.)	+	Neg.	1.0	−	(Neg.)	(Neg.)	+		
2		(Neg.)	(Neg.)		Neg.	0.25		(Neg.)	(Neg.)		<0.1	Neg.
3		(Neg.)	(Neg.)		Neg.	0.5		(Neg.)	(Neg.)		<0.1	Neg.
E.P. 1	+	0.1†	0.005	−	(Neg.)	(Neg.)	−	(Neg.)	(Neg.)	+	0.03	0.5†
2		Neg.	0.015		(Neg.)	(Neg.)		(Neg.)	(Neg.)		<0.1	Neg.
M.D.	+	Neg.	<0.1	+			−	(Neg.)	(Neg.)	+		
H.S.	+	Neg.	<0.03	+	Neg.	0.5	−	(1.0)	(Neg.)	+	0.03	0.5†
A.K.	−	(Neg.)	(Neg.)	+	Neg.	0.5	−	(Neg.)	(Neg.)	+	0.1	Neg.
S.L. 1	+	Neg.	0.02	+	Neg.	0.5	+	<0.1	Neg.	+	<0.1	Neg.
2		Neg.	0.06		Neg.	1.0		<0.1	Neg.		0.03	Neg.
N.O.	+	Neg.	<0.1	+	Neg.	0.5	+	<0.1	1.0†	−	(0.25)	(Neg.)
F.A.	+	Neg.	0.01	−	(Neg.)	(Neg.)	+	<0.1	Neg.	+	0.06	0.5†
N.E.	+	Neg.	0.03	+			−	(Neg.)	(Neg.)	+		
R.G.	+	Neg.	0.06	+			+	0.12	Neg.	+		

Numerals = minimum concentration needed for inhibition (mg/ml). † 10–15% contamination by immunoelectrophoresis. Neg. = no inhibition at 2 mg/ml. (Neg.) = expected negative, found negative at 2 mg/ml. ( ) = unexpected (false) positive.
* Electrophoretic separation.

TABLE 2

TITRATION OF GM 1 (a) AND IN V (a) ACTIVITY OF 7S γ-GLOBULIN AND PAPAIN FRAGMENTS FROM 2 SERA

		Gm 1 (a) (mg/ml)					In V (a) (mg/ml)			
Serum	Fraction added	2	0.5	0.125	0.031	0.0078	2	0.5	0.125	0.031
S.L.	7S	0	0	0	++	+++	0	0	0	+++
Gm 1 (a +)	A & C	+	+++	+++	+++	+++	0	±	++	+++
In V (a +)	A	+++	+++	+++	+++	+++	+	++	+++	+++
	C	+	+++	+++	+++	+++	0	±	+	+++
	B	0	0	0	0	+++	+++	+++	+++	+++
D.A.	7S γ	+++	+++	+++	+++	+++	++	++	++	+++
Gm 1 (a −)	A & C	+++	+++	+++	+++	+++	++	++	++	+++
In V (a −)	A	+++	+++	+++	+++	+++	++	++	++	+++
	C	+++	+++	+++	+++	+++	++	++	++	+++
	B	+++	+++	+++	+++	+++	++	++	++	+++
Control	None	+++					+++			

0 to +++ refers to degree of agglutination.

S. L. Gm 1 (a+) and In V (a+) and D. A. Gm 1 (a−) In V (a−) are shown in Table 2. These demonstrate clearly the separation of the two activities controlled by these two loci and illustrate that fragments A and C behave similarly.

While it has been recognized that Gm 1 activity is found only on 7S γ-globulin and not on any of the other immune globulins,[21, 27, 28] there are no reports as to the location of In V activity. In view of the previously demonstrated cross-reaction of fragments A and C with γ_{1A}, and 19S γ-globulins and Bence Jones proteins, and the present finding of In V activity associated with these fragments, attempts were made to determine if In V activity also resided in these proteins. Examination of a preparation of pure 19S γ-globulin obtained from a pool of 20 donors and a pure γ_{1A} globulin preparation isolated from pooled plasma from 10 donors revealed In V activity to be associated with each of these fractions. Similar results have also been obtained with γ_{1A} myeloma proteins and pathologic macroglobulins by our-

selves[29] and by Harboe, Osterland, and Kunkel.[30] The results with Bence Jones proteins are of particular interest in that they appear to permit more precise localization of In V activity. Figure 1 confirms the existence of two major types of Bence Jones protein[24] each of which cross-reacts with fragments A and C but which are antigenically distinct from each other when tested with an antiserum to 7S γ-globulin. Even when mixed, they do not contain all the antigenic determinant groups present in fragments A and C. Studies with four Bence Jones proteins of Group B and five of Group A indicated that each of the type B proteins possessed In V activity while only one of the five type A proteins was able to inhibit. The latter (Bu) gave a second band on immunoelectrophoresis although it sedimented as a single peak in the ultracentrifuge. The In V activities of these proteins and the In V type of seven of the sera are listed in Table 3. It is likely that most of the sera were In V ($b+$) but sufficient reagents were not available for complete typing.

TABLE 3

INHIBITORY ACTIVITY OF BENCE JONES PROTEINS OF DIFFERENT IMMUNOLOGIC TYPES[24]

Name and diagnosis	Serum In V (a)	BJP, Type B In V (a)	BJP, Type B In V (b)	Name and diagnosis	Serum In V (a)	BJP, Type A In V (a)	BJP, Type A In V (b)
G.L. MM[1] 0	+	+	−	S.I. MM		−	−
T.Y. MM 0	−	−	+	K.L. MM γ	−	−	−
B.O. MM γ	+	+	−	F.E. MM Hypo γ	−	−	−
R.O. MM γ	Aggl.	+	−	B.U. MM γ		−	+
				M.E. MG[2] α_2	−	−	−
02		−	+	01		−	−
04		−	Int +	03		−	−
05		+		06		−	−
08		+		07		−	−
09		+		013		−	
010		−					
012		+					
014		−					
No. Tested		12	6	No. Tested		10	9
No. Pos.		7	3	No. Pos.		0	1
% Pos.		58	50	% Pos.		0	11

1 MM = multiple myeloma and type-0 no spike. 2 MG = Macroglobulinemia (α_2 globulin).

Confirmatory studies with the remaining 13 Bence Jones proteins where appropriate serum samples were not available are also shown in Table 3. Of eight proteins in Group B, five were definitely positive, one intermediate in reactivity, and two were negative. Of five Group A proteins, none showed In V (a) or In V (b) activity. It seems possible that other Type B proteins (especially 010, 014) also had In V (b) activity since only two out of eight Type B proteins were tested. The findings could not be related to the electrophoretic mobilities of the proteins. Although an insufficient number of sera were available for testing, it seems unlikely that these results reflect differences in the In V types of the sera, since differences between the normal proteins and the myeloma proteins in the same serum have also been noted in patients with multiple myeloma.[26, 29, 30]

Discussion.—Complex molecules the size of γ-globulins do not yet lend themselves readily to precise structural analyses similar to those successfully employed in determining the structure of smaller proteins such as ribonuclease[31, 32] and insulin.[33] However, several approaches in recent years have aided in partially clarifying the structure of some of these proteins. Thus, chemically well defined reductive cleavage has provided evidence for the existence of several chains in γ-globulins from a

number of species[34] and has detected differences in the primary structure of various paraproteins and pure antibodies.[34, 35] The less well defined breakdown with enzymes such as papain, trypsin, and pepsin has yielded fragments about one fourth to one third the size of native proteins which are biologically active[12, 13, 19, 36–38] and each of which may contain one or more of the chains making up the native protein.

The results of the present studies on normal human γ-globulins isolated from individuals of different genetic γ-globulin groups (Gm groups) and similar studies on myeloma proteins by ourselves[29] and by Harboe and collaborators[30] present strong evidence that the Gm factors, controlled by two distinct Gm loci, reside in separate parts of the γ-globulin molecule and suggest that the two major genetic loci affect different parts of the protein. Gm 1 activity, which is found only in 7S γ-globulin, resides in fragment B, which is unique to 7S γ-globulin. On the other hand, InV activity, which is also found in the 19S γ and γ_{1A} globulins as well as some Bence Jones proteins, resides in that part of the molecule which carries the determinant groups responsible for the cross-reaction of all of these proteins and also the antibody combining site. While the exact location of the In V activity on these fragments cannot yet be determined, the observation that many Bence Jones proteins of one antigenic type carry In V activity while few of those belonging to the other type are In V-positive can probably not be explained by differences in the Gm types of the sera and suggests that comparison of these two types of proteins and further controlled chemical or enzymatic degradation of these proteins may permit better delineation of these factors, possibly on individual chains of the molecule. The recent finding by Putnam[40] that the peptide pattern of Type B Bence Jones proteins differs from that of Type A proteins and resembles that of 7S γ-globulin more closely is consistent with this. Of particular interest in further evaluating the possible mode of action of these genes is the observation of Harboe *et al.*[30] that in six individuals studied, the In V activity associated with the 7S fraction was similar to that in the 19S γ-globulin.

From the data currently available, it is not possible to determine the exact site of action of each of the genes, since the fragments may well contain one or more of these chains or even parts of chains. Similar studies of these better defined entities have not yet been possible because of the virtually complete loss of all biologic activities during their preparation. However, the excellent correlation between the two genetic factors and a variety of other structural properties of these fragments employed suggest that while they may not be the ultimate genetically defined units they must at least contain them. A similar type of genetic control appears to exist in the case of the hemoglobins,[39] where each chain is controlled by a separate gene, and may also be found in other proteins.

At present, there are no other techniques available to distinguish the various genetically distinct γ-globulins from each other. However, the results obtained with the different types of Bence Jones proteins, as well as preliminary studies employing "fingerprint" techniques,[39] suggest that further controlled enzymatic degradation and analyses may succeed in detecting the chemical basis of the specificity.

Summary.—3.5S fragments prepared from 7S γ-globulins from sera of a variety of Gm types were assayed for their Gm 1 (*a*) and (*b*) and In V (*a*) and (*b*) activities.

These two activities, which are controlled by two distinct genetic loci, were found on different parts of the γ-globulin molecule. In V activity was always recovered with fragments A and C, while Gm 1 activity was always associated with fragment B. In V activity, but not Gm 1 activity, was also associated with 19S γ-globulins, γ_{1A} globulins, and the majority of Group B Bence Jones proteins. All of these proteins cross-react with fragments A and C of 7S γ-globulin. The finding of two activities which are under separate genetic control on different parts of the γ-globulin molecule suggests that these genes may control individual chains of the γ-globulin molecules.

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*ON THE MECHANISM OF COMPLEMENTATION AT THE LEU-2 LOCUS OF NEUROSPORA**

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Two different defective alleles of a gene determining the structure of a specific protein when in the *trans* configuration often complement each other yielding a protein with catalytic activity. This phenomenon has been referred to as inter-allelic complementation[1] and has been observed in diploids,[2] partial diploids,[3] and heterokaryons formed between haploid organisms.[4] Assuming only that mutant alleles which fail to complement each other share a defective segment in interacting structures while those pairs of alleles that do complement each other do not share overlapping defects, it has been possible to map a series of complementing alleles in a linear overlapping array.[1] The surprising feature of most complementation maps is that they can be represented topographically as a straight line. Recently, two exceptions to the usual "endedness" of such linear maps have been reported. Both the *dumpy* locus of *Drosophila*[2] and the rII A cistron of T4 bacteriophage[5] have been shown to be continuous.

In this report data are presented that indicate that the complementing *leu*-2 mutants of *Neurospora crassa* can be mapped in a linear continuous overlapping display. In addition, an interrelationship is demonstrated between complementation of *leu*-2 mutants and mutation at the related but unlinked locus, *leu*-3. Results are presented of an experiment expressly designed to test an hypothesis that relates complementation between *leu*-2 mutants to an interaction between polypeptides coded by the *leu*-2 and *leu*-3 genes in the formation of a polymer (at least a tetramer) with enzymatic activity.

Methods and Materials.—One hundred fifty-eight *leu*-2 mutants (alleles of *leu* 37501 which in turn, is assumed to be an allele of *leu* 8839[6]) were used in the complementation analysis. All were derived from the inositol requiring mutant 89601 by the inositol-less death enrichment procedure of Lester and Gross.[7] Ultraviolet light was the mutagen employed in obtaining all mutants except D268 and D272 which were obtained after treatment of an *inos, ad*-5 (Y152) (adenine requiring) double mutant with 2-aminopurine.

Complementation was tested by superimposing standard loopfuls of conidia of each of the *leu*-2 mutants on agar slants containing synthetic medium supplemented with inositol. Adenine was added whenever one of the strains required it for growth. Complementation was scored after seven days incubation at 30°C. Growth was detected within 24 hr after mixing strains which