

Biological and chemical differences among proteins having reaginic activity

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The reaginic properties of eleven human sera and/or their chromatographic fractions were examined with the use of physicochemical and biological methods. Proteins with reaginic activity were found to be markedly heterogeneous with regard to their elution characteristics from DEAE columns and with respect to their skin-fixing properties. Certain differences were also noted with respect to the degree of sensitivity of these proteins to sulfhydryl splitting agents. It remains to be learned if the heterogeneity demonstrated in these experiments is compatible with the concept that reaginic activity is associated with a single class of immunoglobulins.

In a previous study from this laboratory,¹ five reaginic sera were fractionated by anion exchange chromatography, and differences were noted in the elution characteristics of the proteins with capacity to passively transfer a positive wheal-and-flare reaction. The heterogeneity of the elution characteristics of reagin activity varied, not only from patient to patient when the same test antigen was used, but also from "reagin to reagin" against different test antigens in the same individual. Since IgG was the only detectable immunoglobulin in the first fraction eluted from the diethylaminoethylcellulose (DEAE) column, it was concluded that in these 5 patients at least a part of the proteins that were responsible for the Prausnitz-Küstner (P-K) reaction belonged to that class.

Such heterogeneity of the reaginic proteins, as manifested by their elution characteristics from a DEAE column, has also been demonstrated by other authors.²⁻⁹ The present experiments were performed to extend the previous observations and to learn if this apparent heterogeneity could be demonstrated when tested by other parameters. The results confirm the previously noted obser-

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vations and indicate that reagins are also heterogeneous as regards their skin-fixing properties and their susceptibility to inactivation with sulphhydryl splitting agents.

MATERIALS AND METHODS

Blood was drawn from 11 atopic individuals who had strong reaginic activity to a number of allergens. Each serum was divided into small aliquots and stored at -20°C . until used. Throughout this report, the sera are designated as Nos. 1 through 11.

Antigens

The following pollens (Hollister-Stier Laboratories, Los Angeles) were used throughout the study and contained approximately 600 to 800 PNU per milliliter: (1) *Artemisia vulgaris heterophylla* (California mugwort), (2) *Cynodon dactylon* (Bermuda grass), (3) *Elymus triticoides* (ryegrass or wild rye), (4) *Franseria bipinnatifida* (western ragweed), (5) *Koeleria cristata* (western June grass), (6) *Lolium perenne* (perennial ryegrass), (7) *Salsola kali tenuifolia* (Russian thistle), and (8) *Distichlis spicata* (salt grass). The following additional allergens (Hollister-Stier) were also used: (1) horse dander (1,500 PNU per milliliter), (2) rabbit dander (200 PNU per milliliter), (3) peanut (300 PNU per milliliter), (4) crystallized bovine serum albumin (1.0 mg. per milliliter) (Armour & Company), and (5) house dust extract which was prepared in this laboratory without glycerin.

Quantitative immunoglobulin determinations

Quantitative immunoglobulin determinations were made with the radial diffusion technique described by Mancini and colleagues.¹⁰ Commercially available agar plates (Hyland Laboratories) that contained goat antibody specific for human IgA, IgM, or IgG were employed. IgG, IgA, and IgM proteins used for formation of the standard curve to quantitate immunoglobulin levels were obtained from the same source. The diameters of the precipitin rings were measured after 16 hours at room temperature.

P-K tests

P-K tests were performed as previously reported.¹ Skin tests were graded from 0 to 4+, as indicated in Table I. When blocking experiments were performed, recipient skin sites were pretreated by intradermal injection of various

Table I. Grading of reaginic activity by P-K test

Wheal diameter (mm.)	Erythema diameter (mm.)	Grade of reaction
<5	0	±
5-10	10-15	1+
10-13	20-25	2+
13-15	25-35	3+
>15 and pseudopodia	35	4+

serum fractions. After 48 hours, dilutions of test materials were applied to pretreated sites. After an additional 48 hours, pretreated sites and untreated control sites were tested simultaneously with appropriate antigens.

Anion exchange chromatography

Sera were dialyzed overnight against 0.01M dibasic sodium phosphate prior to separation on DEAE columns by stepwise elution.¹¹ The following buffers were used: (1) phosphate buffer, pH 7.0, 0.01M; (2) phosphate buffer, pH 7.0, 0.025M; (3) phosphate buffer, pH 6.0, 0.1M; and (4) phosphate buffer, pH 5.0, 0.3M (made 0.3M by addition of 0.15M sodium chloride). Fractions were collected and protein determinations measured by absorption at 280 m μ . The peaks were pooled, concentrated by negative pressure to the volume of the original serum sample, and dialyzed against buffered saline. Total protein content was determined by the Folin method¹² and IgA, IgG, and IgM measured by radial diffusion for all fractions. These were then sterilized by passage through Millipore filters, and twofold serial dilutions in buffered saline were prepared to determine the highest dilutions that would elicit P-K activity.

Rechromatography

Fractions from 2 sera eluted from DEAE with the buffer system described above were re-applied separately to other DEAE columns and eluted with a system of 7 phosphate buffers that had a constant pH of 8.0 but which were of increasing molarity. The molarities were 0.005M, 0.01M, 0.02M, 0.035M, 0.05M, 0.08M, and 0.15M. The fractions thus eluted were examined for IgG, IgA, and IgM by radial diffusion, for total protein concentration by Folin determinations, and for reaginic activity by P-K tests.

Mercaptoethanol reduction

Nine of the sera were diluted either 1:3 or 1:5 with triethanolamine-buffered saline, pH 7.3, and dialyzed at 4° C. under nitrogen for 48 hours against fresh or triple-distilled 0.1M 2-mercaptoethanol diluted in triethanolamine-buffered saline. Five sera were also dialyzed against 0.2M 2-mercaptoethanol. All sera were then dialyzed against 0.01M iodoacetamide for 48 hours followed by buffered saline for 48 hours. P-K studies were performed simultaneously on the treated and untreated control samples. DEAE chromatographic fractions prepared from 5 of the sera were similarly reduced and alkylated and examined for P-K activity.

RESULTS

Anion exchange chromatography (DEAE)

Fig. 1 shows the type of DEAE curve usually obtained. Immunoglobulins were demonstrated in the various peaks as follows: peak 1 contained most of the IgG and no detectable IgA or IgM; peak 2 contained traces of IgG and IgA; peak 3 contained most of the IgA and occasional traces of IgG and IgM; and peak 4 contained most of the IgM and occasional traces of IgG and IgA. Through the generosity of Dr. John Fahey, who supplied antiserum to IgD, it

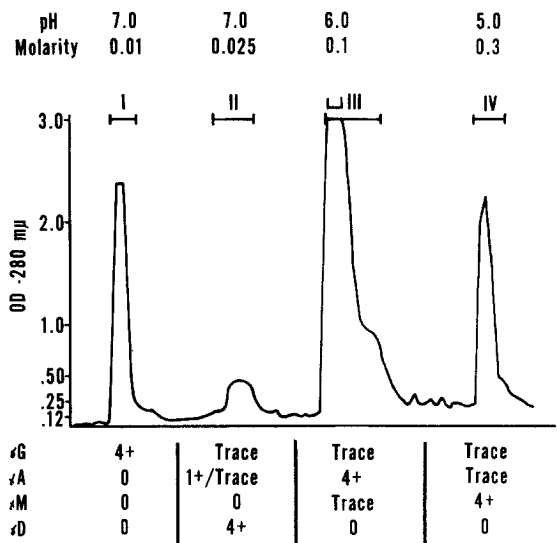


Fig. 1
DEAE curves usually obtained by stepwise elution using phosphate buffers with decreasing pH and increasing molarity.

Table II. Highest dilution of DEAE peaks where P-K activity was 2+ or more

Serum No.	Antigen	DEAE peaks			
		I	II	III	IV
1	Horse dander	1:80	1:80	1:80	0
2	Ryegrass, perennial	1:10	1:10	1:320	0
	House dust	1:20	0	1:320	0
3	Bermuda grass	1:1	1:10	1:10	0
	House dust	1:40	1:40	0	0
4	Ryegrass, perennial	1:20	1:40	1:80	0
	House dust	1:5	1:20	1:20	0
5	BSA	1:5	0	0	0
6	Peanut	1:5	0	0	0
8	Peanut	1:20	1:5	1:10	0
9	Russian thistle	1:2	1:40	1:80	0
10	Ryegrass, perennial	1:10	1:20	1:10	0
11	Peanut	0	1:10	0	0

BSA = crystallized bovine serum albumin.

was also determined that IgD was eluted in peak 2. As shown in Table II, reaginic activity was found in more than one peak eluted from DEAE in 7 of the 10 sera examined in this fashion. In 2 sera (Nos. 5 and 6) reaginic activity was confined to peak 1 and in one (No. 11) it was confined to peak 2. Serum No. 3 was of special interest because the bulk of the reaginic activity to Bermuda grass was in peaks 2 and 3, but most reagin to house dust was in peaks 1 and 2. Serum No. 3 indicated that reaginic activity to different antigens can be found to reside in different peaks in one serum.

To exclude the possibility that the heterogeneity of the elution characteristics

Table III. *Original DEAE peaks 1, 2, and 3 from serum No. 1 after rechromatography*

Molarity of buffers for rechromatography*	Original DEAE peak 1†			Original DEAE peak 2†			Original DEAE peak 3†		
	P-K activity	IgG (mg./ml.)	IgA (mg./ml.)	P-K activity	IgG (mg./ml.)	IgA (mg./ml.)	P-K activity	IgG (mg./ml.)	IgA (mg./ml.)
0.005	2+	5.5	0	0	0	0	0	0	0
0.01	2+	0.1	0	0	0	0	0	0	0
0.02	0	0.1	0	2+	0.1	0	0	0	0
0.035	0	0	0	2+	0.1	0	2+	0.1	0.3
0.05	0	0	0	0	0	0	2+	0.1	0.3
0.08	0	0	0	0	0	0	0	0.1	0.3
0.15	0	0	0	0	0	0	0	0	0

*The pH of all buffers was 8.0.

†IgM was not detected in any fractions tested.

observed in Table II was not the result of contamination by proteins from contiguous peaks, fractions from 2 sera (Nos. 1 and 9) were rechromatographed. On this occasion, the buffers were pH 8 and molarities ranged from 0.005M to 0.15M. The immunoglobulin concentrations and P-K activity of the rechromatographed peaks 1, 2, and 3 from serum No. 1 are indicated in Table III. Reaginic activity from the original peak 1 maintained the same relative elution characteristics with the second buffer system because reaginic activity was again present in the protein fraction eluted with the first 2 buffers where IgG was the only immunoglobulin detected. This consistency in order of elution was also true for the original peaks 2 and 3. Except that some of the P-K activity from the original peak 3 might possibly have been slightly contaminated by trailing from the original peak 2, when peaks from serum No. 9 were rechromatographed, the results were essentially the same.

Heterogeneity of skin-fixing properties of reaginic fractions

Sera from 4 individuals (Nos. 1, 2, 4, and 10), who had reagin in at least peaks 1 and 3, were fractionated into 4 peaks using the stepwise elution system on DEAE (Fig. 1). Fractions from peaks 1 and 3 were selected for this experiment because they were widely separated sources of reagin, and their physicochemical properties would be expected to differ from each other as widely as possible. Sera from 10 normal persons were pooled and similarly fractionated. All normal persons were free of allergic symptoms and had negative skin tests to multiple antigens. Peak 1 of the reaginic and "skin test-negative" sera contained only IgG and peak 3 contained IgA and a trace amount of IgG. Aliquots of "skin test-negative" peaks 1 and 3 were used to pretreat P-K sites and were injected intradermally into suitable recipients. The amount of IgG in the "skin test-negative" peak 1 used for blocking averaged 70 mcg. of N. It was found, however, that as little as 7 mcg. of N was also effective in blocking subsequent fixation of peak 1 reagin. The total protein in the "skin test-negative" peak 3 was higher in that it contained proteins other than IgG and IgA; however, the amounts of IgG and IgA in the material used to block the peak 3 reagin were

Table IV. *Effect on reaginic activity in whole serum of pretreating P-K recipient sites with "skin test-negative" peaks 1 and 3*

Serum No.	Dilution of serum tested	Allergen	P-K reactions at untreated recipient sites	P-K reactions at recipient sites pretreated with "skin test-negative"	
				Peak 1	Peak 3
1	1:640	Horse dander	4+	1+	2+
2	1:640	House dust	3+	1+	2+
4	1:320	Ryegrass, perennial	2+	2+	2+
10	1:320	Ryegrass, perennial	3+	1+	2+

Table V. *The effect on reaginic activity in peak 1 of pretreating P-K recipient sites with "skin test-negative" peaks 1 and 3*

Serum No.	Dilution of peak 1 tested	P-K reactions at untreated recipient sites	P-K reactions at recipient sites pretreated with "skin test-negative"	
			Peak 1	Peak 3
1	1:40	3+	0	3+
2	1:40	3+	1+	2+
4	1:10	2+	0	2+
10	1:10	2+	1+	2+

Table VI. *The effect on reaginic activity in peak 3 of pretreating P-K recipient sites with "skin test-negative" peaks 1 and 3*

Serum No.	Dilution of peak 3 tested	P-K reactions at untreated recipient sites	P-K reactions at recipient sites pretreated with "skin test-negative"	
			Peak 1	Peak 3
1	1:10	4+	2+	2+
2	1:20	2+	0	2+
4	1:10	2+	1+	1+
10	1:1	2+	2+	2+

10 and 40 to 100 mcg. of N, respectively. After 48 hours, dilutions of whole serum or of reaginic peaks were applied to the pretreated sites. The highest dilutions of whole serum or of the reaginic peaks, where P-K activity was 2+ or more, were selected for these studies. Control sites of untreated whole serum or peaks 1 and 3 were also applied simultaneously to the same recipient. Both treated and untreated sites were tested with appropriate antigens 48 hours later.

The effects of pretreating P-K recipient sites with "skin test-negative" peaks 1 and 3 on reaginic activity in whole serum are shown in Table IV. When peak 1 from the "skin test-negative" pool was previously applied to the recipient site, there was partial inhibition in 3 of the 4 sera tested. When peak 3 was similarly applied, there was only slight inhibition of reagin activity in sera Nos. 1, 2, and 10, and no inhibition in serum No. 4. Thus, neither the "skin test-negative" peak 1 nor peak 3 had capacity to completely inhibit the reaginic activity of whole sera.

The effect of "skin test-negative" peaks 1 and 3 on reagin from peak 1 is shown in Table V. When peak 1 from the "skin test-negative" pool was employed as an inhibitor, there was significant partial inhibition of peak 1 reagin in each instance and complete inhibition in 2 cases (sera Nos. 1 and 4 in Table V). When peak 3 from the "skin test-negative" pool was used as an inhibitor for peak 1 reagin, there was only questionable inhibition of the P-K reaction in 1 instance and none in the other 3. These data indicate that "skin test-negative" peak 1 was an effective competitor for the site occupied by peak 1 reagin, but "skin test-negative" peak 3 was not an inhibitor.

The effects of "skin test-negative" peaks 1 and 3 on reagin from peak 3 are presented in Table VI. When peak 1 from the "skin test-negative" pool was the inhibitor, there was some inhibition in 2 instances, partial inhibition in 1, and none in the fourth. When peak 3 from the "skin test-negative" pool was the inhibitor, there was some inhibition in 1 instance, partial inhibition in another, and no inhibition in 2. Therefore, in contrast, it would appear that "skin test-negative" peak 1 was an effective inhibitor of reaginic peak 1 while "skin test-negative" peak 3 is not. On the other hand, neither "skin test-negative" peak 1 nor peak 3 was an effective inhibitor of peak 3 reagin.

Table VII. *Highest dilution of whole serum where P-K activity was 2+ or more before and after treatment with 2-mercaptoethanol and 0.01M iodoacetamide*

Serum No.	Antigen	Highest dilution giving 2+ or more P-K response		
		Before treatment	After treatment with 2-mercaptoethanol	
			0.1M	0.2M
1	Salt grass	1:3	1:3	0
	Horse dander	1:1,280	1:80	0
	House dust	1:160	0	0
2	California mugwort	1:10	0	0
	Bermuda grass	1:80	0	0
	House dust	1:640	0	0
3	False ragweed	1:10	0	0
	House dust	1:80	0	0
	Bermuda grass	1:10	0	0
4	Ryegrass	1:5	1:5	0
	House dust	1:160	1:10	0
	Ryegrass, perennial	1:160	1:20	0
	June grass	1:10	1:10	0
5	BSA	1:64	1:8	0
6	Rabbit dander	1:320	1:80	0
	Bermuda grass	1:40	0	0
7	Bermuda grass	1:320	1:20	0
	Peanut	1:40	0	0
8	Cat dander	1:160	1:10	0
	Ryegrass, perennial	1:20	0	0
	Peanut	1:20	0	0
9	Russian thistle	1:640	0	0

BSA = crystalline bovine serum albumin.

Mercaptoethanol reduction

Table VII demonstrates the effects of reduction of 9 whole sera with 0.1M and 0.2M mercaptoethanol, followed by alkylation with iodoacetamide. In each instance, treatment with 0.2M mercaptoethanol destroyed all demonstrable reaginic activity. However, treatment with 0.1M mercaptoethanol destroyed such activity in only 3 sera (Nos. 2, 3, and 9). Some reagins, such as those reacting with salt grass in serum No. 1 and those reacting with rye and June grass in serum No. 4, appeared to be resistant to treatment by 0.1M mercaptoethanol. Other reagins, such as those directed against all of the antigens studied in sera Nos. 2, 3, and 9, were completely labile to the same treatment. Between these two extremes, some reagins were only partially labile. Furthermore, resistant, labile, and partially resistant peaks with reaginic activity can coexist in a single serum.

To ascertain if these qualities could be a property of one of the reaginic fractions as they are eluted from DEAE, a number of sera were fractionated, and the peaks examined for sensitivity to mercaptoethanol. As shown in Table VIII, partial resistance was present in all fractions that had reaginic activity when there was partial resistance in the whole sera. On the other hand, as exemplified by sera Nos. 2, 3, and 9, when there is complete sensitivity of the

Table VIII. *Highest dilutions of DEAE chromatographic peaks where P-K activity was 2+ or more before and after 0.1M 2-mercaptoethanol and 0.01M iodoacetamide*

Serum No.	DEAE peak or whole serum tested	Antigen	Highest dilution giving 2+ or more response	
			Before treatment	After treatment
1	Peak 1	Horse dander	1:80	1:20
	Peak 2	Horse dander	1:80	1:40
	Peak 3	Horse dander	1:80	1:1
	Whole serum	Horse dander	1:1,280	1:80
2	Peak 1	House dust	1:20	0
	Peak 2	House dust	0	0
	Peak 3	House dust	1:320	0
	Whole serum	House dust	1:640	0
3	Peak 1	House dust	1:40	0
	Peak 2	House dust	1:40	0
	Peak 3	House dust	0	0
	Whole serum	House dust	1:80	0
3	Peak 1	Bermuda grass	1:1	0
	Peak 2	Bermuda grass	1:10	0
	Peak 3	Bermuda grass	1:10	0
	Whole serum	Bermuda grass	1:10	0
4	Peak 1	Ryegrass, perennial	1:20	1:5
	Peak 2	Ryegrass, perennial	1:40	1:10
	Peak 3	Ryegrass, perennial	1:80	1:10
	Whole serum	Ryegrass, perennial	1:160	1:20
9	Peak 1	Russian thistle	1:2	0
	Peak 2	Russian thistle	1:40	0
	Peak 3	Russian thistle	1:80	0
	Whole serum	Russian thistle	1:640	0

reaginic activity to 0.1M mercaptoethanol in the whole serum, the same complete sensitivity was also noted with respect to the isolated fraction.

DISCUSSION

Three types of evidence which demonstrate heterogeneity among molecules with reaginic activity are presented in this report and will be discussed separately.

Elution characteristics of DEAE

Among the 10 sera subjected to chromatography, reagins were isolated in 1 or more individual peaks and sometimes distributed among each of the first 3 of the 4 peaks obtained by stepwise elution. To be sure that this variation in elution was not an artifact of the procedure, the relative purity of the proteins in the original peaks was demonstrated when 2 sera were refractionated on anion exchange columns with a slightly different buffer system. In each case, the proteins with reaginic activity maintained their relative elution characteristics. These data indicate a charge or absorption heterogeneity among the molecules with reaginic activity. While only IgG was found in peak 1, IgG, and IgA, and IgD in peak 2; and IgG and IgA in peak 3, it is possible that other undetected immunoglobulins such as IgE¹³ or IgND¹⁴ were present to account for the reaginic activity in these peaks. Similar results have previously been reported by Perelmutter and colleagues.⁵ In addition, the data presented in this report demonstrate that the reaginic material was eluted in peaks and that there was little or no reaginic activity in the material between the peaks, even when the nonreaginic eluates were concentrated back to original volume. In spite of this, the possibility that all reagins are associated with only one class of immunoglobulins, such as IgE, with a wide elution spread like that described by Ishizaka and Ishizaka¹⁵ has not been ruled out.

Another possible cause for this type of heterogeneity might be explained on the basis of the antigens responsible for eliciting the reagins, analogous to the two types of rabbit IgG immunoglobulins produced in response to antigens of different electrical charge as demonstrated by Sela and Mozes.¹⁶

Skin-fixing characteristics

The experiments involving the inhibition of passive transfer of reaginic activity suggest that at least some of the heterogeneity of reagin is associated with that portion of the molecule which is responsible for skin fixation. The proteins isolated in DEAE peak 3 from "skin test-negative" sera contained detectable IgG and IgA globulins but did not have the capacity to block the subsequent fixation of reaginic peak 3, reaginic peak 1, or whole serum. The failure of peak 3 to be an effective blocker may have been due to the absence of peak 3 blocking material in "skin test-negative pools" or could have been due to a dilution phenomenon during the isolation of peak 3 from the DEAE column. On the other hand, the proteins found in DEAE peak 1 from the "skin test-negative" sera contained only detectable IgG globulin and had the capacity to block the subsequent fixation of peak 1 reagin.

The same material, however, was unable to completely block fixation of the reagin found in whole serum or peak 3 reagin. Since the 4 reaginic sera studied had activity in both peaks 1 and 3, it is not surprising that an individual peak was less effective as a blocker for whole serum than it was for its respective reaginic peak. The observations with "skin test-negative" DEAE peak 1 would suggest the existence of a minimum of two basically different skins fixing moieties on reaginic antibodies in whole serum.

Blocking of reaginic activity, with the use of fractions from normal human serum, was first attempted by Ishizaka and his co-workers.¹⁷ They were able to block the subsequent fixation of reaginic material with fractions rich in IgA (and now thought to contain IgE) but not in fractions rich in IgG. In contrast to their findings, Goodfriend and his co-workers¹⁸ found that fractions rich in IgG but devoid of IgA and fractions with IgG and IgA but devoid of IgM were capable of at least partially blocking reaginic activity in whole serum. However, fractions rich in IgM with trace amounts of IgG and IgA were ineffective when used as blockers of reagins in the Prausnitz-Küstner test. More recently, the same authors have shown that small fragments called 1S and 2S fragments,¹⁹ isolated from IgG-rich fractions, are capable of blocking reaginic activity in whole serum.

Stanworth and his co-workers¹⁴ have effectively blocked the fixation of reagin using myeloma protein, designated by them as IgND, in a P-K test. Thirty mcg. per milliliter was an effective competitor for receptor sites in the recipient's skin. On the other hand, 7S IgG preparations were ineffective. This latter finding would suggest that at least the reagin in the test system used contained similar tissue-binding sites as the myeloma protein. However, it does not exclude the possibility that other types of reagin exist which would not be competitively blocked by IgND.

Susceptibility to alkylation and reduction

Similar to previous reports,^{20, 21} much of the reaginic activity in the 9 sera tested was destroyed by alkylation and reduction with 0.1M 2-mercaptoethanol and 0.01M iodoacetamide. However, some populations of reaginic antibodies in 6 of the 9 sera were partially or even totally resistant to this treatment, and 0.2M 2-mercaptoethanol was needed to completely inactivate these molecules. Reagins to different antigens in individual sera varied in their response to such treatment, some being completely inactivated while others were unaffected or merely reduced in their reactivity. In addition, there was no indication that a given antigen such as Bermuda grass or house dust selectively elicited 2-mercaptoethanol-resistant type antibodies in the group studied. When 2 sera which showed partial resistance to treatment with 0.1M 2-mercaptoethanol were fractionated on DEAE cellulose and the isolated fractions then tested for sensitivity to 0.1M 2-mercaptoethanol, the same partial resistance was noted in each peak. Thus partial resistance to treatment with 0.1M 2-mercaptoethanol was not due to admixture of mercaptoethanol-stable and mercaptoethanol-labile peaks.

These differences in reaginic activity before and after reduction and alky-

lation are another manifestation of heterogeneity of reagins against different antigens in the same individual and also demonstrated heterogeneity of reagin against the same antigen from individual to individual. Unlike the heterogeneity demonstrated by skin-fixing determinants, susceptibility to 0.1M 2-mercaptoethanol seems to show a variation which is completely independent of the absorption and charge properties of the reaginic proteins as they are demonstrated on an anion exchange column.

The immunoglobulins are a heterogeneous group of proteins. This heterogeneity may be considered in two rather broad categories. First, they are heterogeneous as to their physicochemical characteristics. This is manifest in their molecular weight, their size, the unique determinants in the structure of their heavy chains which provide the basis for their classification, and their susceptibility to chemical agents or to heating. Second, they are heterogeneous as to their biological activities. This may be seen by their ability or inability to cross the placenta or to fix complement. Of the 4 well-described classes of immunoglobulins, only IgG freely crosses the placenta.^{22, 23} In contrast to this rather clear association of biological function with the physicochemical characteristics of IgG, some, but not all, IgG and IgM molecules, but apparently no IgA molecules,^{24, 25} are able to fix complement. The data presented in this report describe physicochemical heterogeneity of the proteins which carry with them reaginic activity. It remains to be learned whether reaginic activity is restricted to a single class of immunoglobulins such as IgE or IgND and at the same time has a wide spectrum of physicochemical heterogeneity similar to IgG, or whether the biological function demonstrated as reaginic activity is a property of more than one class of immunoglobulins.

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