

Demonstration of human reagin in the monkey

I. Passive sensitization of monkey skin with sera of untreated atopic patients

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Skin-sensitizing antibody or reagin was first described by Prausnitz and Küstner in 1921.¹ Many attempts have been made to demonstrate this antibody by in vitro techniques such as hemagglutination, precipitation, histamine release, anaphylaxis, and basophil degranulation. To date, the relation of the antibodies detected by these methods to the classical skin-sensitizing antibody in man has been problematical.

Attempts to measure the atopic reagin by passive transfer to monkey skin were first reported by Grove² and were unsuccessful. Harten and associates,³ however, did demonstrate that human skin-sensitizing antibody could fix to monkey skin. A positive reaction was evoked when the antigen was administered orally. Caulfeild, Brown, and Waters⁴ and Straus⁵ also showed that human reagin would bind to the skin of the rhesus monkey. An injection of the specific allergen in the sensitized site led to the development of local edema. Unfortunately these reactions were difficult to reproduce and measure precisely.

Recently, Layton and his co-workers,⁶⁻¹¹ in an important series of articles, have reported on the use of monkey skin as a method for demonstrating the skin-sensitizing antibody in sera of atopic individuals. Reagins against castor bean, pollens, penicillin, and several other antigens have been demon-

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strated. The results seem to be reproducible and to allow quantitation of the skin-sensitizing antibody.

In view of these findings, it was thought advisable to compare this method with the classical passive transfer technique in man. This communication will deal with the specificity of the reaction, the thermolability of the antibody, and the quantitative measurements as determined in both monkey and human skin.

METHODS AND MATERIALS

Antisera

Sera were obtained from patients who were clinically sensitive to ragweed pollen. None of them had received previous ragweed injection therapy. Sera were also obtained from two grass-sensitive patients who were not clinically sensitive to ragweed and from several nonatopic individuals. An antiragweed serum was prepared by injecting monkey "M" intravenously on two occasions with 5 per cent ragweed extract and twice intradermally with ragweed extract emulsified with complete Freund's adjuvant at weekly intervals. Samples of these sera were heated at 56° C. in a water bath for periods ranging from 30 minutes to 4 hours.

Passive sensitization of the monkey skin

The methods described by Layton, Yamanaka, and Denko⁹ were employed with certain modifications. During the course of the following studies several cynomolgus (*Macaca irus*) and rhesus (*Macaca mulatta*) monkeys were used. They varied in weight from 3 to 10 pounds. After removal of the hair with an electric shaver, 0.10 ml. aliquots of various sera were injected intradermally into several sites on the abdomen, thighs, cheeks, and eyebrows of the monkey. These sites were generally marked with red ink. However, the sensitized sites on the chest and abdomen were identified by a grid drawn with a saturated solution of picric acid. Several methods of determining the fixation of this antibody to the skin were employed:

1. Specific allergen (0.10 ml.) was injected locally into each sensitized site from 6 to 48 hours after sensitization. This was then followed by an intravenous injection of 2.0 ml. of 1 per cent Evans blue dye. A positive reaction consisted of a circumscribed blue area within a few minutes due to extravasation of dye at the sensitized site.

2. The specific allergen (2.0 ml.) was injected intravenously after a previous injection of dye.

3. A mixture of both dye and antigen was injected intravenously following previous sensitization of the skin.

The results obtained by these methods were basically quite similar. The direct intradermal injection of antigen, however, was more laborious. In general, the reactions were smaller in size, but this procedure proved to be the most convenient method if many different antigens were to be compared. When the dye was administered prior to intravenous or intradermal injection of antigens, blue spots occasionally developed in areas not sensitized. These reactions were presumably due to trauma.

The simultaneous injections of dye and antigen proved to be the most convenient and reproducible technique and was used in most of our studies. The intravenous injections were easily accomplished by placing a small rubber tourniquet above the knee and using a 22 gauge needle to puncture the saphenous vein. We did not have to resort to surgical cutdowns or intracardiac injections.

Passive cutaneous anaphylaxis (PCA) in the guinea pig

Seven albino guinea pigs weighing 250 grams received intradermal injections of sera from ragweed-sensitive patients. Four hours later 0.50 ml. of a 5 per cent ragweed extract and 0.5 ml. of 1 per cent Evans blue dye were mixed and injected intravenously into three of the guinea pigs. The antigen and dye mixture was injected into the remaining four guinea pigs in a similar manner after 24 hours.

Comparison of skin-sensitizing activity in man and monkey

Serial threefold dilutions of sera from 10 untreated ragweed-sensitive patients were prepared. The quantity of each dilution was sufficient to sensitize each of two nonatopic human recipients, one *Macaca mulatta*, and one *Macaca irus* monkey. Hence, the same prepared dilutions were used for all recipients. Twenty-four hours later, the human volunteers were challenged by local injections into the sensitized sites with ragweed extract and the results were recorded in 15 minutes. The monkeys were challenged with a mixture of ragweed and dye intravenously as described previously and the results recorded.

To compare the thermolability of reagin, samples of three sera from untreated ragweed-sensitive patients were heated at 56° C. for 30 minutes, 1 hour, 2 hours, and 4 hours. The sera were then diluted serially and 0.1 ml. portions of each dilution were injected intradermally into the upper arm of each of two normal, nonatopic recipients, as well as into the skin of a monkey. Twenty-four hours later, each site in the human skin was challenged with a 0.05 ml. injection of 0.5 per cent extract of whole ragweed pollen and the monkey was challenged intravenously with the simultaneous injection of dye and ragweed antigen. The results were read 15 minutes later.

Tanned-cell hemagglutination

The tanned-cell hemagglutination technique was performed with the unheated and heated human sera. Briefly, Group O, Rh-negative red blood cells were treated with 1:25,000 tannic acid and coated with a partially purified fraction of ragweed pollen termed Pool C, which has been isolated and supplied by Goldfarb and Callaghan.¹² Previous experience has shown that the hemagglutination reaction is more sensitive with the use of this material than with whole ragweed extract.¹³ Following washing, these cells were added to serial dilutions of antisera. The patterns were then read after two hours and again after standing overnight at 4° C.

Sucrose gradient fractionation

Sera from two ragweed-sensitive patients were separated by means of the sucrose gradient method and a Spinco Model L preparative ultracentrifuge with

a swinging bucket rotor (SW-39). Sucrose, 10 per cent in buffered saline, was fed into a closed mixing flask containing 4.0 ml. of a 40 per cent sucrose solution. As the inflowing 10 per cent solution displaced the mixture, 4.5 ml. of the latter was layered into each centrifuge tube. A volume of 0.5 ml. of a 1:2 dilution of the patient's serum was added. The samples were then centrifuged at 125,000 g for 15 hours. For sampling, the bottom of each tube was punctured with a 25 gauge hypodermic needle and the samples were collected dropwise in a series of tubes.

RESULTS

Undiluted sera from 7 untreated ragweed-sensitive patients were injected intradermally into duplicate sites on the ventral surface of a cynomolgus monkey. Controls included sera from two atopic patients who were not sensitive to ragweed either clinically or by skin test. In addition, the serum of a normal individual with no allergy was injected into the monkey skin in the same manner. Twenty-four hours later a mixture of 2.0 ml. of 5 per cent aqueous ragweed extract and 2.0 ml. of 1 per cent Evans blue dye was injected intravenously into the monkey. Three to 5 minutes following this injection, circular areas of blue color appeared at certain skin sites injected with the patients' sera. The reactions became more intense over a 5 to 10 minute observation period and reached a maximum at the end of 30 minutes.

Fig. 1 illustrates the appearance of a monkey treated as described above. The diameter of each blue-stained area was measured and rated on a scale ranging from negative to 4 plus. The results of the experiment are recorded in Table I. It can be seen that sera taken from the seven ragweed-sensitive patients all provided strong (2 plus to 4 plus) reactions in the monkey skin. In general, the reactions in the two different sites were identical. None of the three control

Table I. *Passive sensitization of the skin of the cynomolgus monkey with sera from untreated ragweed-sensitive patients*

Patient	Reaction in monkey skin after intravenous injection of ragweed and Evans blue	
	Site 1	Site 2
Ba. (ragweed sensitive)	3+	4+
Hu. (ragweed sensitive)	3+	3+
Ko. (ragweed sensitive)	3+	3+
St. (ragweed sensitive)	3+	3+
Bi. (ragweed sensitive)	2+	2+
Gi. (ragweed sensitive)	3+	3+
As. (ragweed sensitive)	4+	4+
Fi. (atopic—not ragweed sensitive)	—	—
Th. (atopic—not ragweed sensitive)	—	—
Br. (normal control)	—	—

Negative, —

Slight, 1-5 mm. diameter

1+, 5-10 mm.

2+, 10-15 mm.

3+, 15-25 mm.

4+, over 25 mm.

sera provoked any demonstrable reactions. The experiment was repeated with an interval of 48 hours allowed between intradermal sensitization of the monkey and intravenous injection of the ragweed-dye mixture. Identical results were obtained.

Sites for sensitization

In order to make certain that the site on the monkey skin selected for intradermal injection did not greatly influence the results, sera of six ragweed-sensitive patients and one control patient were injected into various locations on the monkey skin. Twenty-four hours later the monkey was injected with ragweed extract mixed with Evans blue dye. After the 30 minute observation period the diameter of the blue-stained areas was measured in the usual manner. The results are presented in Table II. There was some variation in the diameter of reaction in different sites of the monkey skin. For example, serum Av. provided a 4 plus reaction on the skin of the chest and 3 plus or 2 plus reactions



Fig. 1

Passive sensitization of the skin of the cynomolgus monkey with sera from ragweed-sensitive patients. Positive reactions are indicated by dark spots.

Table II. *A comparison of various sites used for passive sensitization with sera from ragweed-sensitive patients*

<i>Patient</i>	<i>Serum dilution</i>	<i>Site*</i>	<i>Reactions</i>
Bi.	1:2	R. cheek	4+
Bi.	1:2	R. mid-abdomen	4+
St.	1:2	R. deltoid	4+
St.	1:2	L. thigh	4+
St.	1:2	R. thigh	4+
Ko.	1:2	R. mid-chest	3+
Ko.	1:2	L. thigh	2+
As.	Undiluted	R. upper abdomen	4+
As.	Undiluted	R. thigh	4+
As.	Undiluted	L. deltoid	4+
Av.	Undiluted	R. thigh	2+
Av.	Undiluted	L. mid-chest	4+
Av.	Undiluted	L. thigh	3+
Gi.	1:2	L. upper eyelid	4+
Gi.	1:2	L. lower abdomen	4+
Fi.	Undiluted (control)	R. upper eyelid	—
Fi.	Undiluted (control)	R. lower abdomen	—
Fi.	Undiluted (control)	L. cheek	—

*R., right.

L., left.

on the thigh. A similar difference is observed with serum Ko. In additional experiments, not contained in Table II, the impression was obtained that the skin of the eyelid gives somewhat greater reactions than other sites of the monkey. However, reactions were very similar on different sites on the anterior chest and abdomen of the animal.

Methods of challenge

In most experiments the ragweed antigen and dye were mixed in the test tube before intravenous administration. When Evans blue dye alone was injected intravenously no reactions occurred at any of the previously sensitized sites. No difference in the size or intensity of dermal reactions was observed whether the dye and antigen were injected together or administered separately.

The reactions obtained by direct intradermal injection of antigen into the sensitized sites were compared with the intravenous administration of antigen. Comparable sites in the skin of a cynomolgus monkey were sensitized with sera from ragweed-sensitive patients. Twenty-four hours later the sites on the right side of the animal were challenged directly with 0.05 ml. of a 0.5 per cent ragweed extract. Ten minutes later 3.0 ml. of a 1 per cent solution of Evans blue were injected intravenously. Within 3 minutes blue areas appeared in the sensitized sites on the right side, reaching a maximum in about 10 minutes. No reactions were visible on the left side at this time. These results are presented in Table III. Twenty minutes later 2.0 ml. of a 5 per cent ragweed extract were injected intravenously. The sensitized sites on the left side now developed blue reactions as indicated in the table. No increase in the size of the reactions on the right side was noted following the intravenous injection of ragweed. The

Table III. A comparison of direct challenge with intravenous injection of antigen

<i>Ragweed-sensitive patient</i>	<i>Dilution</i>	<i>Site</i>	<i>Right side intradermal challenge*</i>	<i>Left side intravenous challenge†</i>
St.	1:2	Lower abdomen	3+	3+
Ko.	1:2	Mid-abdomen	Slight	1+
Gi.	1:2	Upper abdomen	3+	4+
As.	1:2	Upper thigh	3+	4+
Bi.	1:2	Mid-chest	3+	3+
Bi.	1:5	Side	1+	2+
Bi.	1:10	Lower thigh	1+	3+
Th. (control)	1:2	Upper thigh	Negative	Negative

*Sensitized sites injected directly with 0.05 ml. of 0.5 per cent ragweed extract followed in 10 minutes by intravenous injection of dye.

†No direct injection of antigen into sites. Ragweed injected intravenously 10 minutes after intravenous injection of dye. No change in size of reaction on right side occurred after intravenous injection of antigen.

blue spots that appeared on the left side after the intravenous inoculation of antigen were generally larger than the corresponding sites on the right that were directly challenged with antigen. For this reason it was felt that the simultaneous intravenous administration of dye and antigen was generally more satisfactory, and was adopted as the standard procedure.

Specificity of reaction

In order to ascertain the specificity of the passive transfer reaction in the monkey skin the following experiment was carried out. Serum was selected from two allergic patients. Patient Be. was clinically sensitive to grass pollen and showed a marked immediate reaction in intradermal skin tests with grass extract. He also showed a moderate skin reaction to ragweed although he had no history of symptoms during the ragweed season. Patient Gi. was also clinically sensitive to grass pollen and had a strong skin test reaction to grass extract. He showed no clinical ragweed sensitivity and had only a slight skin test reaction to ragweed. Dilutions of these two sera were prepared and injected intradermally into the skin of a monkey. Twenty-four hours later ragweed extract together with Evans blue was injected intravenously. The results are presented in Table IV.

Table IV. Passive sensitization of monkey skin with sera from grass-sensitive patients followed by intravenous injection with ragweed and later grass pollen extracts

<i>Patient</i>	<i>Dilution</i>	<i>Ragweed</i>	<i>Grass</i>
Be.	Undiluted	3+	4+
Be.	1:2	—	3+
Be.	1:6	—	3+
Gi.	Undiluted	—	3+
Gi.	1:2	—	3+

Only the undiluted sample of serum from patient Be. showed reaction to challenge with ragweed extract. Thirty minutes later the same animal was injected with grass pollen extract. Strong reactions were obtained at the sensitized sites. This experiment indicates that serum from patients sensitive to grass pollen sensitizes the skin of the monkey to react predominantly or exclusively with grass antigen. The slight cross-reaction with ragweed extract might well be due to a small degree of sensitivity to this allergen on the part of one of the two patients.

Comparison of PCA in guinea pig to passive transfer in monkeys

The experimental method employed in these investigations has many resemblances to the technique of passive cutaneous anaphylaxis in the guinea pig described by Ovary and Biozzi.¹⁴ The possibility arose that the reactions described above might be attributed to a comparable anaphylactic reaction. An experiment was undertaken to compare passive transfer in the monkey and the guinea pig. Sera from four ragweed-sensitive patients and one nonsensitive control patient were injected intradermally into a monkey. The same sera were injected intradermally into two guinea pigs. In addition, a rabbit antiserum to bovine serum albumin (BSA) and an antiserum to ragweed prepared in a monkey were included. Twenty-four hours later the monkey was injected with ragweed dye mixture and the resultant reactions measured and recorded in Table V. One guinea pig received the same antigen-dye mixture after the 4 hour incubation period customarily used in passive cutaneous anaphylaxis; the second animal was injected 24 hours later. The sera from ragweed-sensitive patients all produced strong reactions in the monkey skin. No blue spots were evident in the skin of either guinea pig. Interestingly, the ragweed antiserum prepared

Table V. *Passive transfer reactions in monkey and guinea pig skin*

<i>Sera</i>	<i>Dilution</i>	<i>Reaction in monkey skin after 24 hours</i>	<i>Reaction in guinea pig skin after 4 hours</i>	<i>Reaction in guinea pig skin after 24 hours</i>
<i>Challenge with ragweed</i>				
<i>Ragweed-sensitive patients</i>				
Ra.	1:5	4+		—
Ra.	1:20	4+		—
Ca.	1:5	3+	—	—
Ha.	1:5	3+	—	
Bi.	Undiluted	2+	—	
Ba.	Undiluted	4+	—	
<i>Nonatopic patient</i>				
Ba.	Undiluted	—	—	—
Antiragweed monkey serum	Undiluted	—	4+	
<i>Challenge with bovine serum albumin</i>				
<i>Antibovine serum albumin rabbit serum</i>				
Unheated	1:5	3+	3+	
Heated at 56° C. 4 hours	1:5	3+	3+	

in a monkey repeatedly provoked a positive reaction in guinea pig but not in the skin of the monkey. Finally, the same animals were challenged by intravenous injection of bovine serum albumin. This injection resulted in reactions in the sites sensitized with the anti-BSA rabbit serum in both monkey and guinea pig. BSA provoked no additional reactions in the other sensitized sites in either animal.

These experiments make it clear that the passive transfer reaction in the monkey is not directly equivalent to passive cutaneous anaphylaxis in the guinea pig. The question then arose as to the similarity of passive transfer in the monkey and the classical passive transfer test of Prausnitz and Küstner (PK test) carried out in the human.

Comparison of passive transfer in man to passive transfer in monkey

The results of skin-sensitizing antibody titration of sera from ten untreated ragweed-sensitive individuals in the skin of two nonatopic humans, a *Macaca mulatta* (10 pounds), and a *Macaca irus* (3 pounds) monkey are presented in Table VI. As will be noted, the 2 human recipients responded essentially equally. There was a one tube difference with only two of these sera. Three sera that produced only weakly positive passive transfer reactions with a maximum dilution of 1:3 failed to sensitize the *Macaca mulatta* monkey. The remaining seven sera, however, produced essentially the same titers in both man and the *Macaca mulatta* monkey.

Thermolability

It has long been known that the reagin responsible for the human reaction is destroyed by gentle heating. For this reason, a series of experiments was carried out to determine the comparative effect of heating of the patients' sera on passive transfer reactions in both species. Serum samples from two ragweed-sensitive patients were placed in a 56° C. water bath for periods of 30 minutes,

Table VI. *Comparison of passive transfer titers in skin of man and monkey*

Untreated ragweed- sensitive sera	Human skin		Monkey skin		
			<i>Macaca mulatta</i>	<i>Macaca irus</i> *	
	Recipient 1	Recipient 2		1	2
Ca.	3†	3	0	0	Not done
Ro.	243	243	243	27	81
Fi.	81	81	81	Not done	27
Hu.	3	3	0	Not done	Not done
Ve.	243	81	81	Not done	0
He.	729	> 729	243	27	81
De.	243	81	81	Not done	27
Ra.	27	27	27		
Ch.	3	3	0		
Hek.	27	27	27		

*The *Macaca irus* monkeys had been used several times previously, whereas the *Macaca mulatta* monkey was used for the first time.

†Reciprocal of highest dilution of serum which still produced positive transfer reaction when challenged with specific antigen.

Table VII. *Effects of heating sera from untreated ragweed-sensitive patients on passive transfer in monkey*

Dilution of patient's serum	Unheated	Heated 56° C.	
		30 minutes	2 hours
Patient Ra			
1:5	4+	4+	—
1:20	4+	3+	—
1:40	3+	3+	—
1:80	3+	2+	—
1:120	3+	2+	—
1:480	—	—	—
Patient Sm			
1:5	4+	2+	—
1:10	3+	1+	—
1:20	3+	1+	—
1:40	2+	—	—
1:80	2+	—	—
1:60	Slight	—	—

Table VIII. *A comparison of the titers of passive transfer reaction in the skin of humans and monkeys and in tanned-cell hemagglutination*

Serum	Unheated			Heated at 56° C.						
				30 minutes		2 hours		4 hours		
	Human Skin	Monkey Skin	HA*	Human Skin	Monkey Skin	Human Skin	Monkey Skin	Human Skin	Monkey Skin	HA
Jr.	240†	120	160	120	120	—	—	—	—	160
Jh.	40	40	320	5	—	—	—	—	—	320
Rs.	80	160	160	5	40	—	—	—	—	160

*Tanned-cell hemagglutination.

†Reciprocal of the greatest dilution giving a positive reaction.

2 hours, and 4 hours. Serial dilutions were prepared and injected into monkey skin. The results are presented in Table VII. Heating of human serum for 30 minutes at 56° C. produced some decrease in the intensity and, in one instance, reduction in the titer of the reaction with ragweed. Heating for 2 hours completely destroyed the reaction of both patients' sera. The sample heated for 4 hours was also completely negative and is omitted from the Table.

In the next experiment samples of serum from three untreated ragweed-sensitive patients were heated under similar conditions. The samples were then divided and one aliquot tested in the monkey in the usual fashion. A second aliquot was injected intradermally into normal non-ragweed-sensitive human recipients. These sensitized sites were tested by intradermal injection of ragweed extract as described in the section on Materials and Methods. A third portion of the same serum was tested in the hemagglutination reaction. The results of these three methods of testing are reported in Table VIII.

Heating sera from ragweed-sensitive patients for even 30 minutes reduces their reaction in passive transfer tests in either human or monkey skin. Com-

plete inactivation of this antibody function was achieved after a two hour heating period. On the other hand, the samples of serum showed no decrease whatever in the titers obtained in tanned cell hemagglutination tests. Thus, a striking parallel is seen between the passive transfer test in monkey and human skin.

Sucrose gradient fractionation

Skin-sensitizing antibody as demonstrated in the PK reaction has been associated with a sedimentation constant varying from 7 to 14S. In order to confirm this observation and compare the PK reagin with the factor responsible for sensitization of monkey skin, three samples of serum from ragweed-sensitive patients were fractionated by sucrose gradient ultracentrifugation. To demonstrate the approximate localization of a "7S antibody," a potent anti-BSA rabbit serum, was simultaneously fractionated by this method. The samples collected were tested for their reaction in monkey skin, and by hemagglutination with ragweed extract or BSA, respectively. The results of one such serum shown in Table IX indicate that maximal activity in the reaginic sera is concentrated in Fractions 5 through 8. The major activity in the anti-BSA serum which represents typical "7S antibody" was concentrated in Fraction 8. It would appear that the skin-sensitizing antibody found in sera from atopic individuals has a sedimentation constant between 7S and 11S. Although several

Table IX. *Skin-sensitizing and hemagglutination activity in sera fractionated by the sucrose gradient method*

Tube No.	Skin-sensitizing activity		Tanned-cell hemagglutination
	Monkey	Human	
<i>Untreated ragweed-sensitive patient Ra.</i>			
1*	—	—	—
2	—	Very slight	—
3	Very slight	Very slight	—
4	1+	Very slight	40
5	2+	2+	160
6	3+	3+	640
7	3+	3+	1,280
8	3+	2+	640
9	Very slight	—	40
10	—	—	20
11	—	—	—
Whole serum 1:10	4+	4+	
<i>Antibovine serum albumin rabbit serum</i>			
1*	—		—
2	—		—
3	—		—
4	—		9
5	Very slight		243
6	Very slight		729
7	Very slight		2,187
8	2+		6,561
9	Very slight		81
10	—		81
11	—		9

*Tube No. 1 represents the sample of more rapidly sedimenting material from the bottom of the centrifuge tube.

different types of serum proteins are known to sediment at these rates, these results exclude the role of a 19S or macroglobulin type of antibody in the reactions measured.

DISCUSSION

The investigations reported in this communication were undertaken as part of a long-range study of methods designed to replace the original passive transfer technique of Prausnitz and Küstner. Our first efforts were directed toward demonstrating skin-sensitizing antibody or reagin in the test tube. These experiments took advantage of cultures of human tissues, including primary cultures derived from human nasal or bronchial mucosa or foreskin, or established cell lines such as strain HeLa. Incubation of these tissues with serum from atopic human patients did not result in any visible change upon subsequent addition of specific allergen.¹⁵ Other investigators have prepared suspensions of leukocytes from the blood of ragweed-sensitive patients.¹⁶⁻¹⁷ Addition of specific allergen produces release of small amounts of histamine that can be measured biologically or by the sensitive fluorometric assay. Van Arsdel¹⁸ has recently reported that leukocytes from nonatopic donors can be sensitized by fresh serum, or serum stored at minus 70°C., from patients allergic to ragweed pollen. The addition of specific antigen promotes a release of histamine in vitro. Shelley¹⁹ has observed that rabbit basophils undergo a process of "degranulation" following incubation with serum from allergic patients and addition of specific allergen. These methods might become available in the future for measurement of human skin-sensitizing antibody.

We have also attempted to demonstrate human reagin in various experimental animals. Like Augustin,²⁰ we were unable to induce passive cutaneous anaphylaxis in the guinea pig with serum of untreated human allergic patients, although we were able to demonstrate that the sera of certain individuals treated either with large amounts of aqueous ragweed pollen extract or by repository injection did provide such reactions.

We were able to confirm the findings of Layton and his co-workers⁶⁻¹¹ that the skin of the *Macaca irus* and *Macaca mulatta* monkeys can be passively sensitized by reaginic sera. We have demonstrated that, without the use of premedication, the simultaneous injection of dye and antigen produces a response in the monkey skin of the same order of sensitivity as in human skin. In one series of experiments it was noted that the *Macaca irus* monkey did not respond as well as did the human or the rhesus monkey. This could be due to the fact that this *Macaca irus* monkey was used for several previous ragweed experiments and could well have developed blocking antibodies, thus inhibiting the reaction. Hemagglutination tests with this monkey's serum revealed relatively high titers on subsequent examination. In a recent review on the properties of the skin-sensitizing antibody, Stanworth²¹ reported on the variability of the reaginic titers in human recipients. It has been shown that they can vary as much as tenfold. Hence, it was not surprising to find that various monkeys of the same size and species may also vary in accepting skin sensitization. If an animal had been used many times, its skin frequently became less responsive.

The question then arose as to whether passive transfer in the monkey measures skin-sensitizing antibody or heat-stable antibody. The experiments in which human sera were heated to 56° C. for various periods of time indicate that the heat stability of sensitizing antibody in the monkey parallels reactions obtained in human skin. Layton¹⁰ reported all skin-sensitizing activity was lost after heating of reaginic serum at 56° C. for only one-half hour. These authors did not do serial dilutions of the sera. In early experiments one of us (C. E. A.²²) reported that human reaginic serum, when tested in human recipients, required heating at 56° C. for 4 hours for complete inactivation. In contrast, hemagglutinating human antibody is not destroyed by heating at 56° C. for as long as 4 hours.²³ Moreover, anti-BSA rabbit serum, after heating at 56° C. for 4 hours, produced a positive dermal response following the intravenous injection of BSA and dye (Table V). Hence the precipitating type of antibody was not destroyed by the heat treatment. For this reason we propose that the reaction in the monkey skin demonstrates reagin and is not simply another method for demonstrating the antibody responsible for precipitation or indirect hemagglutination.

Ultracentrifugation was undertaken in another effort to compare the antibody responsible for sensitizing monkey skin with that sensitizing human skin. Our results substantially confirm previous reports by Augustin and Hayward²⁴ and by Heimlich, Vannier, and Campbell²⁵ that reagin does not sediment as a 19S macroglobulin. The factors responsible for reaction in monkey skin and human skin are identical in their sedimentation properties within the limits of resolution of this method. However, since the naturally occurring hemagglutinin sediments at the same rate, it was not possible to distinguish them from these skin-sensitizing antibodies by sedimentation alone. Nor do these experiments permit us to fix with exactness the sedimentation rate of the skin-sensitizing activity. The data are compatible with a sedimentation constant ranging from 7S to 11S.

With potent antisera the reactivity at different skin sites was approximately the same. With weaker antisera the delicate skin of the eyelids and anterior trunk gave slightly larger blue reactions than did the skin of the thighs.

Up to 54 sites have been sensitized at one time on a monkey. These monkeys could be used repeatedly; we have used one monkey six times. At least two weeks should be allowed between experiments on one monkey. On a few occasions after a one week interval between different experiments, the skin did not react when the antigen and dye were administered 24 hours following sensitization. This could be due to the presence of residual free antigen in the circulation. In our experiments the optimum time interval between sensitization and challenge seemed to be between 24 and 48 hours. However, positive reactions were obtained at varying intervals from 4 hours up to 96 hours. Layton¹⁰ has reported that positive passive transfer in the monkey skin can be demonstrated as long as 14 days after intradermal sensitization.

The advantages of using passive transfer to monkey skin instead of humans are: (1) avoidance of the risk of transferring serum hepatitis; (2) ready availability of recipients; (3) avoidance of delays encountered in culturing sera to insure sterility before injection into a human recipient; and (4) elimin-

ation of the loss of valuable antisera which occurs during filtering procedures. This technique of passive sensitization of monkey skin may prove to be useful in evaluating unknown or potentially toxic allergens or in the standardization of allergens for clinical use.

The effect of injection therapy and the possibility that blocking antibody can be demonstrated in the monkey are under investigation and will be reported in a subsequent publication.

SUMMARY

The technique of passive sensitization of the skin of monkeys is a convenient and reproducible method for the demonstration of skin-sensitizing antibodies. Intravenous challenge with a mixture of antigen and Evans blue dye proved to be technically easier and as reliable as administration of dye followed by antigen. The intravenous administration of antigen generally proved to be superior to the direct intradermal injection with antigen.

Heating of the sera from untreated ragweed-sensitive patients at 56° C. for 2 hours completely destroyed the reaginic activity as demonstrated by passive transfer in the monkey and human skin, while the hemagglutination titers were not changed by heating.

Separation of reaginic sera by ultracentrifugation with the sucrose gradient method suggests that the skin-sensitizing antibody is not a macroglobulin.

This technique for demonstrating skin-sensitizing antibodies in the monkey skin avoids many problems inherent in the classical Prausnitz-Küstner method, including the hazard of transmitting serum hepatitis. It has potential value in evaluating human skin-sensitizing antibody and in the standardization of allergens.

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