

Hypersensitivity responses in monkeys.

IV. Sensitization to human serum and nonacceptance of reagin

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Monkeys immunized with human serum without adjuvant developed high titers of skin reactivity but no precipitins. When skin sensitivity to serum developed, most of the animals failed to accept reagins. The nonacceptors had antibodies which combined with human serum to form antigen-antibody skin-reacting complexes, blocked reagin attachment, produced reverse passive transfer reactions in sites preinjected with human serum, and gave histamine release from human leukocytes or normal monkey leukocytes pretreated with human serum. Passive transfer of these antibodies to normal monkeys was questionable. Such actively immunized monkeys had no greater skin reaction than normal monkeys with myeloma IgE. Serum from sensitized monkeys which still accepted reagins did not disclose similar antibodies. Monkeys immunized with myeloma IgE in complete Freund's adjuvant produced good precipitin titers, no immediate but delayed Arthus-type skin reactions, and they lost their ability to accept reagins. These findings indicate that loss of reagin acceptance is probably due to sensitivity to IgE, that the antibody to human IgE in the monkey is probably not in monkey IgE, and that myeloma IgE and normal human IgE may possess some dissimilar as well as common determinants.

Skin sensitivity to human serum was noted by us about 3 years ago in the first two rhesus monkeys which had lost their ability to accept human reagins after several previously successful reagin transfers.¹ These observations raised the question whether such nonacceptance is due to sensitization of the animal to a serum component, what this component is, and what evidence could be obtained for the involvement of antibodies. It was therefore planned to conduct investigations into the chronological relationship of sensitization and nonacceptance of reagin and to examine a number of immunologic aspects in such animals. These included the following: skin and P-K reactivity to fractions of human reaginic serum; the skin reactions of normal monkeys to complexes of human serum and

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immune monkey serum; the ability of the immune monkey serum to neutralize the reaginic capacity of human serum when mixed in vitro or when preinjected; the capacity of the monkey serum to react on a normal monkey skin site pretreated 24 hours earlier with human serum (reverse passive transfer); the ability of the immune serum to release histamine from human leukocytes or from monkey leukocytes pretreated with human serum; and the question of acceptance of human reagins after an animal had been immunized with purified IgE (myeloma).

MATERIALS AND ANIMALS

Reaginic human serum (RHS) was obtained from 4 individuals (Bad., Asd., Ril., and Ste.) allergic to ragweed pollen, with the following initial P-K titers in monkeys, respectively—1:625, 1:125, 1:125, and 1:125. The same serum was always used in a complete comparative experiment. Fractions of one of the sera (Asd.) were prepared for us by Dr. K. Ishizaka. Nonreaginic human serum (NHS) was obtained from one donor (Lee).

Ragweed pollen extract was an extract of giant and short ragweed in concentrations usually of 1:33 w/v for intravenous challenge and 1:1,000 or 1:300 for intradermal challenge. Other dilutions were used also for testing sensitivity.

Monkey serum was of three types: serum from animals which had not been subjected to injections of any antigen (NMS), serum from animals that had developed skin reactivity to human serum and a failure to accept reagins (0 accept S), and serum from animals which had developed such skin reactivity but still accepted reagins (+ accept S). For skin titrations fivefold serial dilutions of serum were usually employed.

Monkey leukocytes for in vitro histamine release investigations came from the same three classes of monkeys. Human leukocytes were obtained from one of the subjects (Ste.) from whom reaginic serum was procured.

IgE (courtesy of Dr. K. Ishizaka) was from a myeloma subject and was used in complete Freund's adjuvant for immunization of monkeys and other animals. For challenging and for in vitro investigation two- and fivefold serial dilutions of IgE were used.

Polybrene (hexadimethrine hydrobromide), obtained through the courtesy of Dr. Floyd McIntire, Abbott Laboratories, was used in a 1 per cent solution.

Evans blue dye (Warner-Chilcott, Morris Plains, N. J.), 0.5 per cent, was given intravenously in 3 to 4 ml. amounts.

Twenty-two monkeys were used for various experiments. Twenty were *Macaca mulatta*, and 2 *Macaca speciosa*.

PROCEDURES AND RESULTS

Relation of skin sensitivity to human serum and nonacceptance of reagin

Evans blue dye was injected intravenously 15 minutes prior to intradermal or intravenous challenges. Capillary permeability to human serum in unsensitized animals was determined by intradermal injections of fivefold dilutions of serum. Bluing resulted from dilutions ranging from 1:1 to 1:25. As far as possible the same reaginic serum was used in the same animals, but because of depletion of sera, some exceptions had to be made.

The majority of animals subjected to several series of injections of reaginic serum from passive transfer experiments developed inability to accept reagins (Table I, Figs. 1 and 2). All of these also developed a skin sensitivity to human serum, but some animals that had a titer of skin sensitivity to human serum of 1:125 or higher dilution still accepted reagins. Of particular interest are those monkeys, shown in Table I, which were deliberately sensitized to human serum.

Table 1. *Relation of skin sensitivity to human serum and acceptance or non-acceptance of reagin*

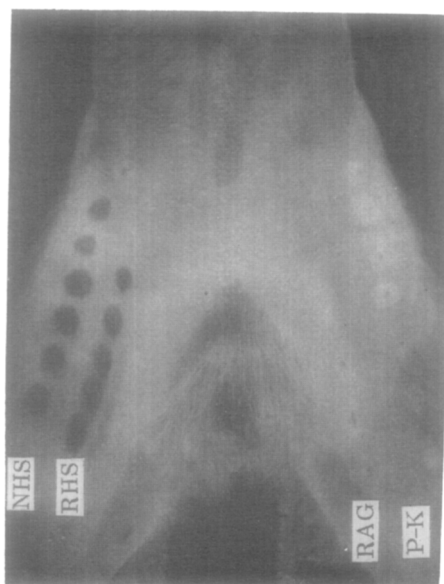
Monkey No.	Nonacceptance		Acceptance	
	Initial reagin titer	Highest serum dilution giving skin reaction	Representative titer during plus-acceptance	
			Reagin	Highest serum dilution giving skin reaction
1	625	78,125		
2	625	125		
3	625	15,625		
4			125	125
5	125	15,625	25	78,125
6			25	625
7	625	3,125		
8	625	3,125		
9		10,000	25	125
10	125	125	25	625
11	125	125	125	125
13		15,625	5	625
14	5	625		
15			25	625
16		625		
17	25	125		
18	625	625		

In Tables I through V the end points are expressed as reciprocals. Of the 3 animals, Nos. 5, 10, and 11, which accepted reagins despite skin sensitivity to human serum and on which we have subsequent data, all lost their ability to accept reagins later, and in 2 acceptance of reagins returned still later. Animals Nos. 5, 9, 10, 11, and 13 are listed in columns of both acceptors and nonacceptors because of the change in reactivity.



Fig. 1

Right side of normal monkey. Outer column shows capillary permeability reactions from normal human serum (*NHS*). The three reactions, reading from the bottom upward are from *NHS* 1:1, 1:5, and 1:25. The medial row shows *P-K* reactions (*Asd. serum*) from 1:1,000 ragweed intradermally in sites possibly sensitized with serum 1:1, 1:5, 1:25, and 1:125. Tests were made on Sept. 6, 1968.

**Fig. 2**

Same monkey as in Fig. 1, tested Feb. 12, 1969. The first column on right side of the animal shows reactions to normal human serum (*NHS*) from 1:5 to 1:15, 625. The second column shows direct skin reactions from reagenic serum (*RHS*) 1:5 to 1:3,125. On the outside of the left side of the monkey the column of sites, sensitized with the reagenic serum 24 hours previously, were challenged intradermally with ragweed 1:1,000 (*P-K*). None of the sites reacted (0 accept). On the left medial side are negative reactions to ragweed (*RAG*) 1:3,000, 1:1,000, and 1:300 in unsensitized areas.

After the titer of a ragweed reagin was determined, 2 animals (Nos. 7 and 14) were given 25 ml. of nonreagenic serum intravenously, followed by another 25 ml. 24 hours later. At 7 days the P-K titer (Bad.) remained at the original level of 1:625 and 1:5, and the skin reaction to serum (from normal capillary permeability) was 1:25 and 1:1, respectively. At 12 and 8 days, respectively, the P-K titers were negative, and end-point reactions to human serum were 1:625 and 1:125, respectively. Subsequently, the titers of skin reactivity to serum increased to 1:3,125 and 1:625. Passive transfer of Fractions II₁ and II₁₁ (reagin-rich fractions prepared by Ishizaka) gave results similar to those with whole reagenic serum.

One animal (No. 8) was injected intradermally with 0.2 ml. of normal human serum at several-day intervals. After 3 injections (Days 0, 4, 7) and 13 days following the initial injection, the animal reacted to human serum in a dilution of 1:125 and failed to accept ragweed reagin. After 8 months the skin reactivity to human serum was 1:3,125, and there was no acceptance of reagin.

One monkey (No. 3) with a serum capillary permeability of 1:1 received 5 ml. of normal human serum intravenously and another 5 ml. 24 hours later. After 19 days the skin sensitivity to serum was 1:125. After several doses of 1 ml. subcutaneously, the titer had not increased 14 days later. Several more injections were given intradermally. The titer 2 weeks later was 1:15,625. How-

Table II. *P-K titer of serum fractions (Ishizaka) using Monkey Nos. 17 and 18 as recipients*

<i>Fraction</i>	<i>Protein content (mg. of N/ml.)</i>	<i>Monkey No. 17 P-K titer</i>	<i>Monkey No. 18 P-K titer</i>
I	2.7		
II ₁	0.77	125	625
II ₁₁	0.49	125	125
III	1.1	0(2)	0(2)
IV	2.3	0(2)	0(2)
V	2.7	±(2)	0(2)
VI	1.88	±(2)	0(2)
Whole reaginic serum		25	25

Monkey No. 17 was challenged with ragweed 1:1,000 intradermally and Monkey No. 18 with 3 ml. ragweed 1:33 intravenously. The protein content (determined by Dr. K. Ishizaka) is the amount of protein nitrogen of the undiluted solutions. The parentheses indicate use of diluted material. Thus 0(2) means the ragweed challenge was negative in a site of 1:2 dilution.

Table III. *Titer of skin reactions from complexes of human serum with monkey serum*

<i>Beaginic human serum dilutions (final)</i>	<i>Monkey No. 17</i>		<i>Monkey No. 18</i>		
	<i>0 accept S</i>	<i>NMS*</i>	<i>0 accept S</i>	<i>NMS*</i>	<i>+ accept S</i>
3,125	0	0			
625	0	0	+	0	±
125	+	0	+	0	0
25	+	0	+	0	0
5	+	0	+	0	±
2	++	++	++	0	++

*NMS = normal monkey serum.

ever, P-K reactivity was lost earlier at the beginning of serum (Bad.) sensitivity.

Monkey No. 4, which had a serum capillary permeability titer of 1:5 and a P-K titer (Bad.) of 1:625, received 2 injections of 5 ml. of normal human serum intravenously and developed a skin titer of 1:25 after 19 days. Two months later, after a number of subcutaneous and intradermal injections, the skin titer to serum was 1:125 and the P-K titer was 1:125.

Two immunized animals (0 accept) gave a skin reaction to 8 and 200 ng. of myeloma IgE, respectively. A third immunized animal failed to react to 200 ng. Of the animals sensitized to serum and still accepting reagins, one reacted to 40 ng. of IgE, while the other did not react to 200 ng. Of 2 normal animals, one reacted to 200 ng. and the other failed to react to that dose.

P-K and antigenic activity of serum fractions

The P-K titers of the fractions of human ragweed reaginic serum were determined by intradermal injections of fivefold serial dilutions in normal monkeys and by challenging the sites 24 hours later with ragweed pollen extract 1:1,000 intradermally or 3 ml. of ragweed extract 1:33 intravenously. Fractions II₁ and II₁₁, containing only a small portion of the globulins in whole serum, were as active as the whole serum. It is apparent that on the basis of globulin weight these fractions are at least 50 times as potent as the whole reaginic serum

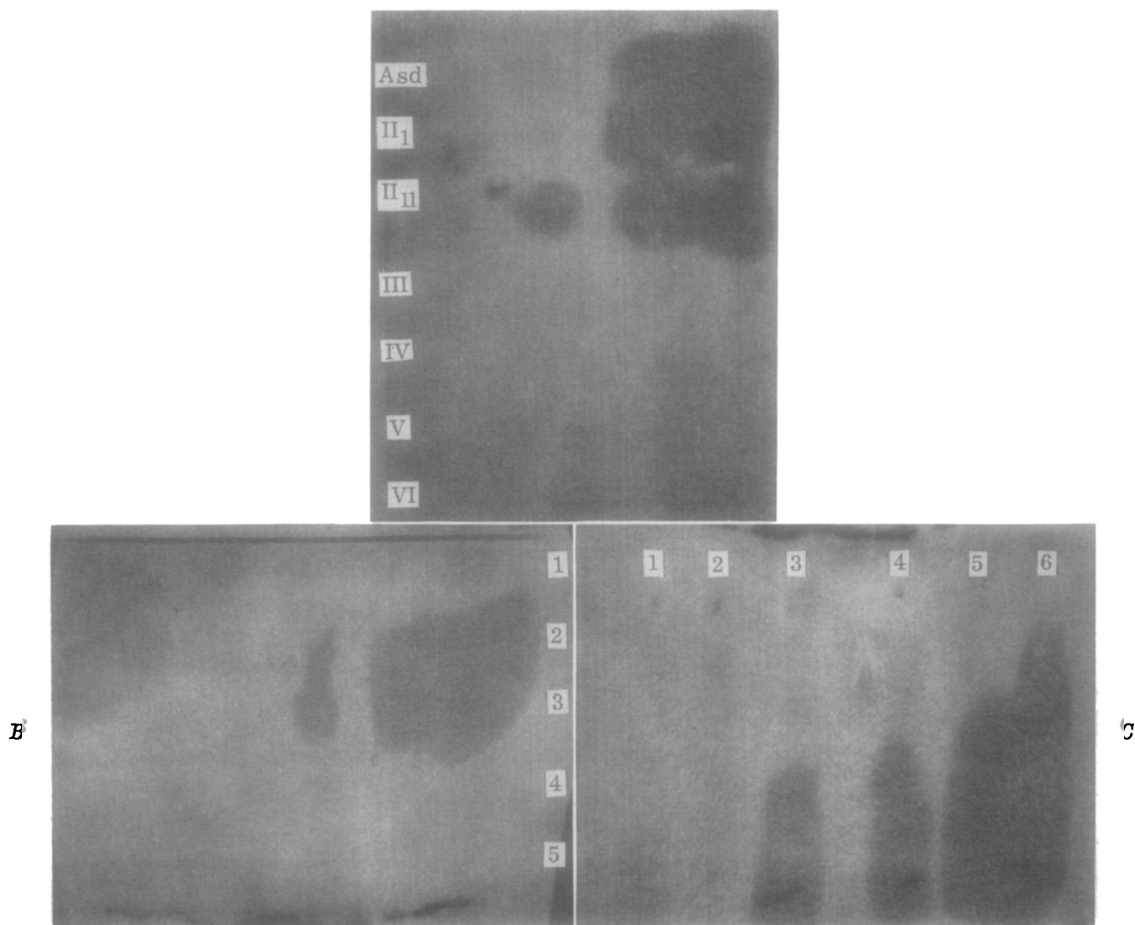


Fig. 3

A, Reactions on the chest of a normal monkey (No. 18) after intravenous injection of 3 ml. of mixed ragweed pollen extract 1:33. This animal had been sensitized 48 hours previously with reaginic serum and with fractions from that serum prepared by Dr. K. Ishizaka. The titrations were made horizontally from monkey's left toward right, in dilutions of 1:5, 1:25, 1:125, 1:625, 1:3,125 for whole serum and Fractions *II*₁ and *II*₁₁. An additional 1:2 dilution was used for the other fractions. Note that in addition to the whole reaginic serum only Fractions *II*₁ and *II*₁₁ had a discernible reaginic titer, and the titer was at least as high as in the whole reaginic serum. *B*, In vitro neutralization of reagin (same animal as in *A*). Titrations were made horizontally on the chest and abdomen beginning with the strongest on the left side of the animal. Row 1 had been injected intradermally 48 hours earlier with mixtures containing equal parts of 0 accept monkey serum and dilutions of reaginic serum to make the final dilutions of the latter 1:2, 1:5, 1:25, 1:125, and 1:625. Row 2 was treated similarly except that + accept serum was used instead of 0 accept serum. Row 3 had similar preparations, using normal monkey serum instead of serum from immunized animals. Three milliliters of ragweed extract 1:33 intravenously produced no reactions in Row 1, indicating that the reaginic globulin had been neutralized by the 0 accept serum, while Rows 2 and 3 gave reactions, indicating that the + accept serum was not capable of neutralizing the reaginic globulin. Rows 4 and 5 had been injected with dilutions of nonreaginic human serum and normal monkey serum. The absence of reactions in these sites served as additional control tests. *C*, In vivo neutralization of reagin (same animal as in *A*). Intradermal injections were made in vertical columns. Columns 1 and 2 were injected with 0 accept serum 1:5, Columns 3 and 4 with normal monkey serum 1:5, and Columns 5 and 6 with + accept serum 1:5. Two hours later, reaginic serum (Asd.) in fivefold dilutions beginning with 1:5 at the bottom were injected in Columns 1, 3, and 6. Fraction *II*₁₁ in comparable dilutions was injected in Columns 2, 4, and 5. Ragweed intravenously 48 hours later gave no reactions in the columns treated with 0 accept monkey serum (Columns 1 and 2), indicating that the latter blocked the reagin, while the columns pretreated with normal monkey serum or + accept serum reacted.

Table IV. *In vitro* neutralization of reagin by immune monkey serum

Reaginic human serum dilutions (final)	Monkey No. 17		Monkey No. 18		
	0 accept S	NMS	0 accept S	NMS	+ accept S
625			0	0	0
125			0	0	0
25			0	+	+
5	0	+	0	+++	+++
2	0	++	0	+++	+++

Complexes were those injected as described in text for Table III. Reactions shown are those with ragweed 1:1,000 intradermally 24 hours later in Monkey No. 17 and with 3 ml. of ragweed 1:33 intravenously in Monkey No. 18, 48 hours later.

(Table II and Fig. 3, A). The fractions and human serum albumin were tested intradermally in monkeys sensitized to human serum. Albumin gave no reaction. All the globulin fractions gave positive skin reactions.

Complexes of human serum with monkey serum

As shown in Table III, fivefold serial dilutions of ragweed reaginic human serum (Asd., titer varying from 1:5 to 1:125) were incubated for 2 hours at 37° C. with equal parts of serum from a monkey (No. 14) with a 1:125 titer of skin reactivity to human serum, which had failed to accept reagin (0 accept S). Preparations of reaginic serum with normal monkey serum were made. The mixtures were injected intradermally into Monkey No. 17. Similar preparations were made with serum from Monkey No. 11 (0 accept S) with a skin reactivity titer to serum of 1:125, Monkey No. 10 (+ accept S) with a skin reactivity titer of 1:625 and a P-K titer of 1:25, and normal monkey serum. These mixtures were injected intradermally into Monkey No. 18. The preparations containing the 0 accept serum with reaginic serum (titer 1:125) reacted in one animal, and 0 accept serum with reaginic serum (titer 1:625) in the other, while the preparations containing the + accept serum reacted only in concentrations attributable chiefly to the normal capillary permeability of the sera.

In-vitro neutralization

As shown in Table IV and Fig. 3, B, 24 hours following the injection of sites described in the tests with the complexes and the injection of additional sites with serial dilutions of nonreaginic serum and normal monkey serum, all sites were challenged intradermally in one monkey (No. 17) with ragweed extract 1:1,000. The other monkey (No. 18) was challenged in 48 hours with 3 ml. of ragweed extract 1:33 intravenously. In both animals the sites containing the reaginic serum with the 0 accept serum, normal monkey serum only, and nonreaginic serum only showed no reactions from the ragweed. The sites containing the mixtures of reagin and normal monkey serum or + accept serum gave reactions. This indicates that the 0 accept serum has an antibody which blocks or neutralizes the reaginic antibody (IgE?) but that the + accept serum does not have an antibody against reagin (or has less), despite the fact that this monkey was skin sensitive to human serum.

Table V. *In vivo neutralization of reagin by immune monkey serum***A. Monkey No. 17**

Dilutions	0 accept S		+ accept S		Buffered saline	
	RHS	II ₁₁	RHS	II ₁₁	RHS	II ₁₁
3,125	0	0	0	0	0	0
625	0	0	0	0	0	0
125	0	0	0	0	0	0
25	0	0	0	+	0	++
5	0	0	0	++	++	+++

B. Monkey No. 18

Dilutions	0 accept S		+ accept S		NMS	
	RHS	II ₁₁	RHS	II ₁₁	RHS	II ₁₁
625	0	0	0	0	0	0
125	0	0	+	±	0	±
25	0	0	+++	++	++	++
5	0	0	+++	+++	+++	++

Monkey sera 1:5 injected intradermally followed 2 hours later with reaginic human serum (RHS) or Fraction II₁₁. Monkey No. 17 was challenged 24 hours later with ragweed intradermally and Monkey No. 18, 48 hours later with ragweed intravenously.

In vivo neutralization

As shown in Table V and Fig. 3, *C*, one monkey (No. 17) was given intradermal injections of 0 accept monkey serum in 2 columns of 6 sites in each and + accept serum in 2 similar columns. Two other series of sites were injected with buffered saline. Two hours later one of the 0 accept, + accept, and buffered saline columns was injected with ragweed reaginic serum and the others with Fraction II₁₁,* prepared from a ragweed reaginic serum. Ragweed 1:1,000 was injected intradermally in each site 24 hours later. The control sites (buffered saline plus ragweed reagent) reacted. The 0 accept sites failed to react, indicating that this serum has antibodies which neutralize or block the human reagent. The + accept sites reacted where Fraction II₁₁ was injected but not where the whole reaginic serum had been injected.†

The other animal (No. 18) was prepared as No. 17, except that normal monkey serum was used in place of buffered saline. The ragweed challenge was an intravenous injection of 3 ml. of 1:33 ragweed pollen extract. The results were definite: negative in the 0 accept columns and strongly positive in the sites treated with + accept or normal monkey serum.

Reverse passive transfer

Even though direct passive transfer of the 0 accept serum was usually unsuccessful, it is still possible that the serum has antibodies which do not fix to monkey skin cells but are nevertheless capable of inducing a skin reaction when injected into a site where human serum (IgE) is fixed to the skin. As shown in Table VI and Fig. 4, two monkeys were injected intradermally in columns with serial dilutions of reaginic serum and Fraction II₁₁. Twenty-four

*Fraction II₁₁ was no longer available at this point.

†In this experiment the control P-K did not react higher than 1:5.

Table VI. Reverse passive transfer: Reactions to monkey sera in skin sites pretreated with human reaginic serum dilutions

A. Monkey No. 17

Dilutions	P-K RHS			P-K II ₁₁		
	0 accept S (1:5)	0 accept S heated (1:25)	+ accept S (1:5)	0 accept S (1:5)	0 accept S heated (1:25)	+ accept S (1:5)
3,125	0	0	0	0	0	0
625	++	0	0	++	0	0
125	++	0	0	+++	++	0
25	++	+	0	+++	++	0
5	++	+	0	+++	++	0
2	++	+	0			

B. Monkey No. 18

Dilutions	P-K RHS				P-K II ₁₁	
	0 accept S (1:5)	0 accept S heated*	+ accept S (1:5)	+ accept S heated*	0 accept S (1:5)	+ accept S (1:5)
3,125	QNS†		0		±	0
625	++		0		++	0
125	++		0		++	0
25	++		0		++	0
5	++		0		++	0

Reaginic human serum or Fraction II₁₁ was injected intradermally in dilutions indicated. Intradermal challenge was made 24 hours later with indicated dilutions of monkey sera.

*These columns were not challenged because the capillary permeability from the heated monkey serum alone was 1:125 in this animal.

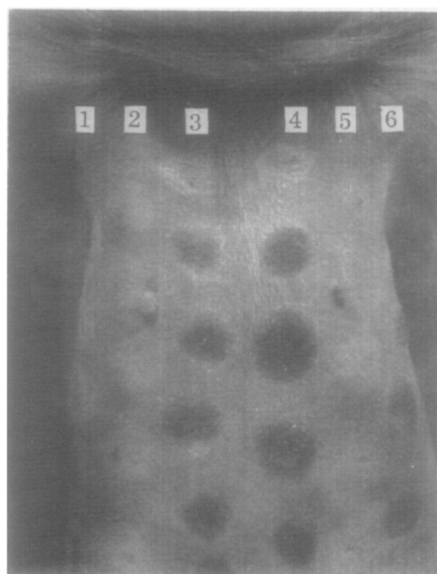
†QNS = quantity not sufficient.

hours later the sites on Monkey No. 17 were challenged with 0 accept serum, + accept serum, and 0 accept serum which had been heated for 2 hours at 56° C. The 0 accept sites reacted in high titer, 1:625, while the + accept sites failed to react. In all instances the dilution of monkey serum used for challenge was one which failed to give a capillary permeability reaction on control sites. In this monkey the heated serum gave a higher capillary permeability reaction, and therefore a greater dilution of serum was used for challenge, which explains the lesser reaction on reverse passive transfer. Monkey No. 18 also was challenged with 0 accept and + accept monkey serum and gave results similar to those of Monkey No. 17. However, the capillary permeability from the heated 0 accept and + accept sera themselves occurred at such a high titer (1:125) as to make the challenging impracticable.

In vitro histamine release

In vitro histamine release studies were done with the use of the method of May.² This method was chosen since only 10 ml. of blood is required and drawing large amounts of blood from monkeys is not feasible. One per cent Polybrene³ was used to separate leukocytes since it was found that dextran, as used by Lichtenstein and others for the separation of human leukocytes for in vitro histamine, was not adequate for monkey leukocytes. Both human and monkey leukocytes were handled in an identical manner.⁴

Cells were separated, washed twice in a tris buffer containing human serum

**Fig. 4**

Reverse passive transfer (Monkey No. 17). On the right side of the chest, 3 columns of 6 sites each were injected with reaginic human serum in dilutions, reading upward, of 1:2, 1:5, 1:25, 1:125, 1:625, and 1:3,125. The left side of the animal was injected similarly with fivefold serial dilutions of Fraction II₁₁, beginning with 1:5. The sites were challenged 24 hours later, as follows: Columns 1 and 6 with heated 0 accept serum 1:25, Columns 2 and 5 with + accept serum 1:5, Columns 3 and 4 with 0 accept serum 1:5. Note that both the heated and unheated 0 accept sera gave reverse passive transfer reactions, while the + accept serum failed to do so.

Table VII. *In vitro* histamine release

<i>Preparation</i>	<i>Antigen or antibody dilution</i>	<i>Histamine released (%)</i>
0 accept L	RHS, 1:5	82
+ accept L	RHS, 1:5	0
NML	RHS, 1:5	0
NML + RHS	0 accept S, 1:45	80
NML	0 accept S, 1:5	10
NML + RHS	+ accept S, 1:5	11
NML + RHS	NMS, 1:5	16
NML	+ accept S, 1:5	11
NML + (+ accept S)	RHS, 1:5	8
NML + (0 accept S)	RHS, 1:15	77
RHL	0 accept S, 1:5	51
RHL	+ accept S, 1:5	0
RHL	NMS, 1:5	0

0 accept = animal not accepting reagin; + accept = animal accepting reagin; L = leukocytes; N = normal; M = monkey; RH = reaginic human; S = serum.

albumin, suspended in the same buffer containing also calcium and magnesium ions,⁵ and then added to antigen or antibody dilutions before being incubated at 37° C. for one hour. A modification of the fluorometric method of Shore and associates⁶ was employed for the actual histamine assay with the use of the Aminco-Bowman spectrophotofluorometer. Passive sensitization of the cells

was accomplished as suggested by Lichtenstein and Osler⁷ with the use of 10 per cent serum concentrations.

The preparations involved are listed in Table VII. Although the results are reported as single determinations, repeat experiments were made and were consistent with the findings presented here. Monkey leukocytes from an animal which had failed to accept reagin (O accept L) or normal monkey leukocytes (NML) exposed to O accept serum produced a high percentage of histamine release with human reaginic serum. Reaginic human leukocytes (RHL) or normal monkey leukocytes pretreated with reaginic human serum (RHS) gave good histamine release when challenged with O accept serum. All experiments involving sera from monkeys which still accepted reagins and all other control experiments gave 0 to 16 per cent release.

Nonacceptance of human reagin in monkeys immunized to IgE

Two monkeys were immunized with myeloma IgE in complete Freund's adjuvant. The first monkey was given 2 injections of 100 μ g 4 weeks apart. No precipitins or skin sensitization (in solutions as concentrated as 20 μ g per milliliter) were obtained. It was suspected that the preparation of IgE might have lost its antigenic activity. With a new batch of IgE this monkey and another were given two doses of 1 mg. in complete Freund's adjuvant, divided into footpads and intramuscularly, 4 weeks apart. Two weeks later the serum of both monkeys showed precipitins in high titer, and immediate skin reactions were elicited with 200 ng. It should be noted, however, that capillary permeability was obtained in normal monkeys with the same amount of the new batch of IgE. While neither myeloma IgE nor whole human serum gave a significant immediate reaction, both produced a 24 hour delayed reactivity in high titer (IgE 0.06 ng.; whole serum 1:625, approximately 0.04 ng. of IgE). These reactions appeared to be of the Arthus type. Sites passively sensitized with human reaginic serum failed to react on challenge with ragweed extract.

DISCUSSION

A number of investigators,⁸⁻¹² using monkeys for experiments with human reagins, have noted that after a variable number of such injections the animals failed to accept reagins. Rose and associates⁸ were inclined to attribute this phenomenon to the development of blocking antibodies to the antigen (ragweed). Buckley and Metzgar⁹ say that ". . . a monkey can be safely used only once for such studies because of potential sensitization to human serum proteins. . . ." Layton¹⁰ states that "after about six weeks or two months they can no longer be sensitized by human reagin: This refractory state will last for six months or so. Then they can be re-used once provided that the time between the sensitization and challenge is only 24 hours or so. . . ." Our experience indicates that once the animal becomes nonaccepting it remains so practically indefinitely (as long as 2½ years) in most instances. Only 2 of the nonaccepting animals were able to accept reagins later. However, it is possible that this persistence of nonacceptance and sensitization to human serum may be due to the booster

effect of the testing at intervals. Patterson and associates¹¹ noted that a month after the injection of human reagins the monkeys failed to accept reagins, and the monkeys were also sensitized to human serum. In a later report they¹² noted that 9 days after intravenous injection of reaginic serum there was an abrupt disappearance of acceptability of reagin.

In our planned investigations with sensitization by the intravenous route, the time of onset of nonacceptance (8 to 12 days) agrees with that of Patterson and associates, while with sensitization by intradermal injections the occurrence of nonacceptance may have been more delayed, but the degree of serum sensitization was greater. We also have the impression that sensitization by the subcutaneous route is not as effective.

Patterson and associates¹¹ were able to transfer human serum sensitivity from one monkey to another. We reported similar findings.¹ However, the results of later trials of passive transfer from monkey to monkey were questionable. Like Patterson's group, we found no precipitating antibodies in animals sensitized intravenously or intradermally. However, as Weiszer, Patterson, and Pruzansky⁴ found, we have also noted that in spontaneous sensitivity of monkeys (to *Ascaris*), the antibodies transfer well to monkeys. This indicates perhaps that the latter antibodies may be in the IgE fraction of the monkey serum (which would fix to skin cells), while the antibody from induced human serum sensitivity may be in another fraction, such as IgG.

Both human serum and monkey serum produce capillary permeability reactions in nonsensitive monkeys. This varies with the batch of serum and the animal tested and must be determined for each experiment. Such reactions with human serum occurred usually in dilutions up to 1:5 or 1:10 and with monkey serum from 1:1 to 1:5. Higher and lower levels were also found. In all animals in which nonacceptance of reagin developed, a skin sensitivity to human serum of 1:125 was adequate for this effect. However, some of the animals which did not lose reagin acceptance nevertheless had a high titer of skin sensitivity to serum. For example, Monkey Nos. 14 and 11, which lost their ability to accept reagins, had a titer of skin sensitivity to human serum of 1:125, while Monkey Nos. 5 and 10 gave passive transfer to reagins in titers of 1:5 and 1:25, despite their much higher skin sensitivity to serum of 1:15,625 and 1:625, respectively. Sera from these 4 monkeys were used in most of the experiments, and the findings point to a definite qualitative difference in antibodies between the two types of sera.

The results of the experiments with the antigen-antibody complexes, in vitro and in vivo neutralization, reverse passive transfer, and in vitro histamine release indicate that circulating antibodies to human serum components are present in the serum of monkeys which develop an inability to accept reagins. These antibodies can combine with human serum antigens in vitro to form complexes which give skin reactivity. They can neutralize the reaginic activity of the human serum and can block or neutralize the human serum reaginic IgE when injected 2 hours previously. The antibodies also react with the human IgE fixed to the monkey skin 24 hours after injection of human serum (reverse passive transfer). This resembles the observation of the Ishizakas¹³ that a reverse

skin reaction can be produced in man by the intradermal injection of precipitating rabbit or guinea pig antiserum to human IgE. They were also able to elicit reactions by the injection of such sera into the skin of normal monkeys.

The results from *in vitro* histamine release clearly show that the animals which do not accept reagins have an antibody to human serum on the leukocytes as well as in the serum. However, these *in vitro* histamine release experiments do not prove that the antibodies involved are directed against IgE. While it is true that in many instances these histamine releases required a high concentration of human or monkey serum to produce them, nevertheless the control experiments were clearly negative or within the accepted normal figures.

All these findings suggest that the monkeys not accepting reagins have developed antibodies to human IgE, while those still accepting reagins have failed to develop such antibodies, at least in comparable quantity. The inference is strong that the loss of acceptance of reagin is due to the development of high sensitivity to the IgE component of human serum. It is possible, of course, that antigen-antibody systems responsible for the skin reactions and for skin-reactive complexes may be different from those involved in neutralizing reagin, blocking of passive sensitization, and reaction with human IgE. Also the possible participation of anti-light chain antibodies cannot be excluded.

The failure of the animals to develop skin reactivity to human serum albumin is understandable, since it has been shown¹⁴ that the albumin of rhesus monkeys and man are virtually identical; this is not true of the globulins.

The role of IgE sensitivity in the production of nonacceptance of reagin is further strengthened by the fact that animals sensitized with myeloma IgE in complete Freund's adjuvant lose reagin acceptance. These animals have a high precipitin titer and no immediate skin-reacting titer, but they do have delayed skin reactions (Arthus) with myeloma IgE and human serum. One must assume, therefore, that both precipitating and nonprecipitating antibodies to IgE will prevent reagin acceptance, though this may represent merely a quantitative difference.

Some as yet unresolved discrepancies in our findings are present. Animals sensitized with myeloma IgE in complete Freund's adjuvant gave a delayed (Arthus) reaction to 1 ng. of myeloma IgE and to a dilution of whole serum containing approximately the same amount of reaginic IgE. On the other hand, in nonacceptor monkeys sensitized to human serum without adjuvant and giving immediate skin reactions to high dilutions of the serum (1:15,000), the maximum amount of IgE in the serum giving the reaction could only be 0.002 ng. The same animal required as much as 8 to 200 ng. or more of myeloma IgE to produce an immediate skin response.

Four alternative possibilities suggest themselves to explain this apparent discrepancy. One is that IgE sensitization is not responsible for the development of nonacceptance of reagin. It could be speculated that the intense skin reaction from the other serum globulins removes or destroys the reaginic IgE. Arguing against this supposition are the results in several of our experiments in which the serum from a nonaccepting monkey behaves specifically against nonmyeloma IgE, while the serum from an acceptor, even though originating from an animal

as highly sensitive to whole serum, fails to give reactions against the nonmyeloma IgE. A second possibility is that the myeloma IgE and reaginic IgE have different antigenic determinants. Third, the possibility has to be considered that the antibody to IgE responsible for the development of nonacceptance is neither an antibody giving a visible skin reaction or necessarily a precipitating antibody. And finally nonacceptance may be accounted for by anti-IgE antibodies, but some of the confusing findings may result from the concomitant presence of other antibodies not directly related to the nonacceptor state but which may be present in high titer. Further investigation is needed for answers to these questions.

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