to a non-specific anti-inflammatory action.

The suppression of established delayed hypersensitivity by methotrexate may provide a theoretical basis for the use of drugs which suppress immune responses in treatment of established diseases thought to be autoimmune in nature, since these disease states may be related to the delayed hypersensitive response(12). Methotrexate has already been successfully employed to inhibit established experimental allergic encephalomyelitis(3) and auto-immune thyroiditis(4), both experimental diseases often considered to be autoimmune in nature, and to be related to delayed hypersensitivity.

Since the effect of methotrexate appears to be on cell multiplication rather than to be a direct cytotoxic phenomenon(6,13), the observation that delayed hypersensitivity may be suppressed after treatment with methotrexate for as short a period as 15 days strongly suggests that the life span of the effector cells involved in the tuberculin response in guinea pigs must be considerably shorter than this period. Methotrexate has no effect on passively established, but non-adoptive delayed hypersensitivity(14), possibly because no cell multiplication is necessary for expression of this reactivity. The recurrence of tuberculin hypersensitivity in actively sensitized guinea pigs after cessation of methotrexate administration could have been due to the persistence (during methotrexate administration) of cells which had been sensitized to tuberculin, but which had been prevented from multiplying by methotrexate, or, alternatively, to resensitization of the animal by the heat-killed B.C.G. still present.

Summary. Actively or passively established tuberculin hypersensitivity in Strain 2 guinea pigs is suppressed by methotrexate treatment for as short a period as 15 days. The suppression of this immune response does not seem to be due to a lymphopenic or anti-inflammatory effect of the drug.

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Cutaneous Anaphylactic Reactions with Antibodies of Different Qualities.*† (29283)

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An abundance of data exists concerning the quantity of antibody required to elicit various immunologic reactions, but considerably less is known about the various qualities of antibodies needed for these purposes. Antibody taken from hyperimmunized animals has been used almost exclusively in studies

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of passively induced immunologic reactions. This antibody, having a relatively high affinity may behave differently from the relatively low affinity, first response antibody responsible for various lesions such as those of serum sickness. Experiments were therefore designed to test the ability of antibody, having a relatively low affinity for antigen, to provoke anaphylactic reactions. Related experiments have been reported by Osler(1), in which antisera, partly absorbed with antigen, were found to be less efficient in bringing about anaphylactic and Arthus reactions. By contrast, however, Weinrach and Talmage concluded that antibody with low affinity to red blood cells, induced lysis with complement more efficiently than did high affinity antibody(2).

Materials and methods. Antigen. Bovine serum albumin (BSA) (Armour) 5 × crystallized was employed throughout. BSA was trace-labelled with I^{131} (I*) according to a method described elsewhere(3). Control tests showed that the I* activity was over 99% protein bound as indicated by precipitation in 10% trichloracetic acid. Electrophoresis of the I*BSA in agar showed that 97% of the activity migrated in the albumin peak and over 98% of this activity could be precipitated with anti BSA. These results indicated that I* activity truly represented BSA. Other control tests have shown that in the presence of half saturated ammonium sulfate, under conditions similar to those in the present study, there is neither further binding nor dissociation of antigen and antibody(4, 5).

Antibody. Antibody to BSA (anti BSA) was obtained following repeated courses of BSA in 0.15 M NaCl in rabbits. Animals were bled 3 days following antigen elimination from the circulation. Antibody having relatively low affinity for antigen was prepared by serial absorption of pooled anti BSA with small amounts of antigen. Eighteen absorptions were performed so that the resulting antiserum failed to precipitate with small amounts of antigen in liquid medium or in double diffusion tests in agar (for details, see 6). This antiserum was called the absorbed antiserum. Antigen could not be detected in the absorbed antiserum upon addition of strongly precipitating antibody, indicating that the absorbed antibody was not complexed with antigen. For comparison, pooled antibody from the same rabbits was employed. It was treated with an unrelated washed immune precipitate once (human gamma globulin-HGG-anti HGG, 200 μg precipitated antibody N per ml of pooled anti BSA). This comparative antiserum was called the unabsorbed antiserum. The globulin fraction of both antisera was then obtained by fractionation in 50% saturated ammonium sulfate. The unabsorbed antiserum was diluted with normal rabbit globulin (also absorbed with HGG-anti HGG) so that the 2 preparations of antibody contained approximately equal quantities of antibody nitrogen (see below).

The quantity of absorbed, non-precipitating antibody was measured by co-precipitation (for details see 6), while the unabsorbed precipitating antibody content was determined by the quantitative precipitin test(7). Three ml conical tubes were employed and reaction volumes were maintained below 0.6 ml to minimize any effect of dilution. Two different pools of precipitating antisera with precipitin levels of over 2.0 mg antibody N/ml were employed to co-precipitate the antibody in the absorbed antiserum. No significant difference between the two was noted. The values obtained were 308 μg co-precipitating antibody N/ml and, after dilution with normal globulin as noted above, 300 μg precipitating antibody N/ml. The capacity of the two antisera to bind antigen was tested using the ammonium sulfate technique(4). With a concentration of 0.08 μg N BSA/ml being used as the combining antigen, the calculated "antigen combining capacity" (ABC) values obtained were 18.4 for the absorbed and 49.5 for the unabsorbed antisera.

Rates of association and dissociation of antibody with antigen. Rates of association of antibody were determined by the method of Talmage(5). I*BSA was mixed with antibody in the cold and immediately, and at various intervals of time thereafter, duplicate samples were removed and added to an equal volume of saturated ammonium sulfate. The

concentration of antigen was 0.08 μg nitrogen/ml and the antiserum was diluted so that 18% of the antigen was bound after an 18-hour incubation period. This required over a 1:3000 dilution of the antiserum, an essential feature of the ability of this technique to measure association rates. It should be noted that in the cutaneous anaphylactic tests as employed herein, (see below), considerable dilution of immunologic reactants also existed. Dissociation rates were measured according to the method of Farr(4): I*BSA was combined with the antiserum and allowed to equilibrate for 24 hours at room temperature. Then a great excess of unlabelled antigen was added (300 molecules unlabelled BSA per labelled molecule) to replace preferentially any dissociated labelled antigen. At that moment and at various intervals thereafter, the amount of I*BSA still bound to the antibody was determined by precipitation in one-half saturated ammonium sulfate. The concentration of I*BSA employed was 0.1 μg N/ml and a dilution of antibody was employed so that 20% of the I*BSA was bound. Both in the dissociation and association rate tests, the amount of I*BSA precipitated with normal serum in 50% saturated ammonium sulfate was less than 5% of the maximal experimental values.

Separation of 7S and 19S antibody. Density gradient ultracentrifugation with a sucrose gradient of 10 to 37% was employed (8).

Passive cutaneous anaphylaxis. The method of Ovary was followed(9). Albino male guinea pigs weighing 300-350 g were used throughout for the anaphylactic tests.

Radioimmunoelectrophoresis. This procedure was carried out by separating the absorbed and unabsorbed anti BSA in the ordinary microimmunoelectrophoresis preparation (10). To the troughs was then added sheep anti rabbit gamma globulin along with a small amount of I*BSA containing 100 μg N/ml, having a specific activity of approximately 2 μc/μg N. After processing and staining the slides in the usual manner, they were placed against ordinary projection photographic plates for 5 days. The photographic plates were then developed and the resulting lines compared to those on the stained immunoelectrophoretic slides.

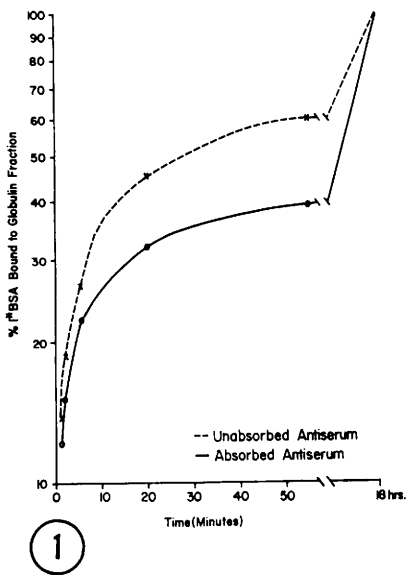

FIG. 1. Association rates of I*BSA with absorbed and unabsorbed anti BSA.

Results. Rates of association and dissociation of the specifically absorbed and unabsorbed antisera. The results of these analyses are presented in Fig. 1. A lag in the speed of association of absorbed antiserum was observed. While, in both cases antibody combined rapidly with I*BSA, by 20 minutes the absorbed antiserum had bound distinctly less antigen than the unabsorbed. From this time, a difference of a few to many minutes existed between the time required for each antiserum to attain that particular level of binding. For example, the absorbed antiserum required 20-30 minutes longer to attain a level of 35% binding. Test of dissociation rates (Fig. 2) showed that the antigen dissociated more readily from the absorbed than the unabsorbed antiserum. Thus, the antigen associated more slowly with and dissociated more rapidly from the absorbed antiserum than the unabsorbed antiserum. This indicated that the absorbed antiserum exhibited a relatively low affinity for the antigen.

Passive cutaneous anaphylaxis with low and high affinity antisera. Passive cutaneous anaphylactic (PCA) reactions were carried

TABLE I. Passive Cutaneous Anaphylaxis in Guinea Pigs with Rabbit Absorbed and Unabsorbed Anti BSA.*

		Diameter of reactions (mm)									
		Absorbed antiserum (μg Ab N/ml)				Unabsorbed antiserum (μg Ab N/ml)				Norm rabbit globulin† (Dilution)	
BSA I.V. (mg N)	Guinea pig No.	30.0	3.00	.30	.03	30.0	3.00	.30	.030	1:10	1:100
.80	1	25	15			22	14			trace	0
	2	23	13			28	14			0	0
	3	21	14			22	15			0	0
	4		18	14	6		14	12	7		
	5		13	7	0		11	7	0		
	6		15	10	0		13	10	0		
	7		12	7	0						
	8		17	12	6						
Avg		23	14.7	10	–	24	13.5	10	–		

* All injections of antibody I.D. in 0.1 ml 3 hr prior to administration of BSA and 0.5 ml of 1% Evans' Blue I.V.

† Normal pooled rabbit globulin prepared, as the 2 antibody preparations, by fractionation with ammonium sulfate at 50% saturation. Dilutions made in 0.15 M NaCl so that amount of globulin used was equal to that in the 2 highest antibody concentrations.

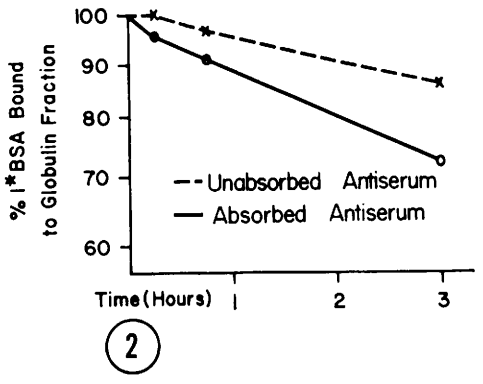

FIG. 2. Dissociation of I*BSA from absorbed and unabsorbed anti BSA.

out using an excess of antigen for challenge, in order to find whether the absorbed, low affinity antiserum was as capable as the unabsorbed, high affinity antiserum in eliciting these reactions. The 2 antibody preparations were found to provoke nearly equal PCA reactions in the *excess of antigen,* showing approximately the same effect of dilution (Table I). This strongly suggested that on the basis of equal weight of antibody the "fixation" to the tissues as well as anaphylactic potency of the two antisera was the same. To compare the 2 antisera under more stringent conditions, the amount of challenging antigen was markedly reduced so that 0.200, 0.020, 0.010 and 0.003 mg BSA N were injected intravenously along with Evans' Blue to guinea pigs 3 hours after 0.3 μg Ab N of each antiserum had been given intradermally. The most notable finding of this experiment was a strikingly slow development of PCA reactions using the absorbed, low affinity antiserum, and in some of the animals, the poor reactions noted when they had reached a maximum. The reactions occurring at 5 and 20 minutes after antigenic challenge are listed in Table II. The slower and poorer reactivity

TABLE II. Effect of Lowered Antigen Concentration on Passive Cutaneous Anaphylaxis with Absorbed and Unabsorbed Anti BSA.

		Diameter of reactions (mm)*			
		Absorbed antiserum		Unabsorbed antiserum	
BSA I.V. (mg N)	Guinea Pig No.	5 min.	20 min.	5 min.	20 min.
.200	1	10	11	11	11
	2	10	10	10	10
.020	3	±†	14	11	11
	4	—†	13	12	16
.010	5	—	13	12	14
	6	—	12	12	12
	7	—	—	±	10
	8	—	8	15	15
	9	±	14	15	17
	10	±	14	13	14
.003	11	—	12	12	12
	12	±	12	10	17
—	13	—	—	—	—
—	14	—	—	—	—

* 0.3 μg Ab N injected I.D. 3 hr prior to challenge.

† ± denotes trace blueing visible at periphery at 5 min. This gradually filled in to give solid reaction as recorded. — denotes a negative reaction.

was noted only when 20 μg N or less antigen was injected. Since it had been determined that the absorbed antiserum would not bind the antigen as well as the unabsorbed, under conditions of considerable dilution, PCA reactions were repeated using 4 times the amount of absorbed antiserum, or enough to yield almost double the amount of binding of the high affinity antiserum *in vitro*. Six guinea pigs were tested with this increased dosage of absorbed antiserum and the previous dosage of unabsorbed antiserum intradermally. After 3 hours the animals were given 10 μg N BSA and Evans' Blue intravenously. They showed no observable change in speed of development of the PCA reaction with the larger dosage of absorbed antibody, and only a slight enlargement of the reaction at 20 minutes. Thus, even when more of the absorbed, low affinity antiserum was injected than the high affinity antiserum, the slower rate of development of the anaphylactic reaction was still observed.

Attempts to find if the antibody molecules in the 2 antisera sedimented differently were carried out employing density gradient ultracentrifugation. Antibody in the 19S and 7S zones of the gradient was examined by both PCA and antigen binding tests. The binding test showed that over 95% of the antibody in both the absorbed and unabsorbed sera was in the 7S region of the gradient. The small amount of antibody activity in the sediment may have represented aggregated gamma globulin containing antibody. In addition, PCA tests with 200 μg nitrogen antigen showed that equal amounts of antibody activity were contained in the 7S region of both antisera. In addition, radioimmunoelectrophoretic analysis of the 2 antisera demonstrated that the antibody in both migrated in the γ_2 region. Differences in migration were not discernible between the two.

Complement fixation by the two antisera was tested using 3 μg antibody nitrogen of each serum, 0.6 μg BSA nitrogen and 106 50% hemolytic units ($C'H_{50}U$) of guinea pig complement. Fixation of complement was carried out at 4° for 18 hours. The results showed that the low affinity, absorbed antiserum inactivated 22 $C'H_{50}U/\mu$g antibody nitrogen and the high affinity antibody approximately 29 $C'H_{50}U/\mu$g antibody nitrogen, after correcting for the small amount of complement fixed by each antiserum in the absence of nitrogen.

Conclusions. These results indicate that a 7S γ_2 rabbit antibody having a low affinity for its antigen was equally as efficient as a 7S γ_2 high affinity antibody in producing cutaneous anaphylaxis in guinea pigs when abundant antigen was present. However, when the concentration of antigen in the reaction was reduced the antibody of low affinity was not as potent as the antibody of higher affinity. A possible explanation of the inefficiency, marked by a slow rate of development and at times less severe anaphylactic reaction, was found to lie in a relatively slower rate of association noted between the absorbed antiserum and its antigen (Fig. 1). This slower rate of association may well bear causal significance when considering the finding that when one merely increases the concentration of antigen in the circulation, the rate of anaphylactic response of the slowly associating antiserum was increased to equal that of the control, rapidly associating antiserum (Table I). These latter conditions favored more rapid and complete binding of the lower affinity antiserum by antigen. These findings fail to support the possibility that the absorbed, slower associating antibody was less capable of "fixing" to tissues or for other reasons less capable of reacting, since merely by raising the dosage of antigen it became fully reactive *in vivo*. Other tests failed to show significant differences between antibodies in the 2 antisera: both were 7S, γ_2 globulins as determined by density gradient ultracentrifugation and radioimmunoelectrophoresis. When mixed with antigen they both fixed abundant hemolytic complement. The antigen-antibody dissociation rates of the 2 antisera were not appreciably different considering the amount of time required to demonstrate as much as 15-20% difference. Further, in other unpublished studies in this laboratory, results similar to those herein reported have been found using anti BSA obtained from individual rabbits after primary immunization and absorbed once with a small

amount of antigen. This diminishes the possibility that the results could be explained by some unique quality of the absorbed antiserum.

The role played by low affinity antibody in immunopathologic lesions may be considerable. In serum sickness the antibody responsible is without much doubt of relatively low affinity. In this disease alone such diverse lesions as endothelial proliferation, increased glomerular permeability, vascular necrosis and anaphylactic alterations in vessels take place. Anaphylactic reactions bringing about an increase in vascular permeability may be of special importance in causing circulating antigen-antibody complexes to localize in vessel walls to produce the lesions. Anaphylaxis may thus act as a trigger mechanism in initiating the development of the disease(11-13). The extent to which low affinity antibody may influence the development of related lesions has not been determined. These studies, along with those of Osler(1), indicate that low affinity antibody may be less efficient in precipitating anaphylactic responses under conditions of low concentrations of antigen. However, when an optimum of antigen is present such antibody is fully reactive.

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Massive Diuresis Following Renal Homotransplantation.* (29284)

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With awareness that massive diuresis must be added to severe oliguria as a potential hazard following sudden relief of urinary tract obstruction, it has become apparent that observed large urine volumes may be the common expression of diverse pathogenetic mechanisms. Wilson, Riseman and Moyer reported acute shocking salt loss following prolonged diuresis(1), and persistence of a salt wasting defect over many months was meticulously characterized by Bricker and coworkers(2). However, a vasopressin resistant water diuresis was responsible for polyuria in the case studied by Earley(3), while solute diuresis principally due to urea has recently been recorded by Maher, Schreiner and Waters(4). In this case, urea comprised 37-56% of urine osmolality during an 8-hour period of observation in which concentrations of serum urea nitrogen (SUN) and serum creatinine (SCr) fell from 175 to 75 and from 17.8 to 7.4 mg per 100 ml respectively. The authors speculated that a similar phenomenon might account for the sometimes massive diuresis seen following renal homotransplantation into azotemic subjects.

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