like activity for *Streptococcus faecalis, Lactobacillus casei* and sulfonamide-treated *Escherichia coli.* The activity of the steroid differs from that of thymine but is of the same order of magnitude.

We are indebted to Lederle Laboratories Division, American Cyanimid Company for the supplies of pteroylglutamic acid and pteroylglutamic acid antagonists used in this study.

Received May 29, 1950. P.S.E.B.M., 1950, v74.

On Hemolysis Mediated by Non-Erythrocytic Antigens, Their Homologous Antibodies and Complement. (17972)

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Kondo(1) observed hemolysis in mixtures of sheep erythrocytes, suspensions of *Serratia marcescens,* and fresh guinea pig serum. He attributed lysis to the enhancement of bacterial hemolysin by guinea pig complement. A recent brief note by Fisher and Keogh(2) described the lysis by complement of erythrocytes which had adsorbed bacterial components and anti-bacterial antibodies. The observations of these authors have been extended in the present report.

Materials and methods. (1) *Antigens.* Trichloroacetic extracts(3) were prepared from *Salmonella typhosa* O 901 and from *Escherichia coli,* strain H. After dialysis against water, the extracts were concentrated to contain approximately 0.5 mg solids per ml, sodium chloride was added to obtain isotonicity, the reaction was adjusted to pH 7, and sterility was attained by Seitz filtration. In another experiment human plasma, in place of bacterial antigens, was used.

(2) *Antisera and complement.* Seitz filtered, heat-inactivated immune sera from four rabbits were employed. One of each of the immune sera was prepared by immunization with *E. coli, S. typhosa,* human serum, and washed sheep erythrocytes. For the detection of rabbit serum adsorbed on the surface of erythrocytes, a chicken immune serum against rabbit euglobulins, absorbed with rabbit serum albumin and pseudoglobulin, was used. Aliquots of immune sera, employed in some experiments, were freed of normal hemagglutinins by double absorption at 37°C (two 15 minute periods) with 0.5 ml packed erythrocytes for each ml of serum. The fresh pooled serum from at least 3 normal guinea pigs, diluted 10-fold in saline, served as complement.

(3) *Erythrocytes* and *"sensitization."* Erythrocytes from sheep blood preserved in Alsever's solution, rabbit cells from fresh defibrinated blood, and human type O cells from citrated blood were used. The cells were usually washed twice in saline, and a 1% suspension was then prepared, using as suspending fluid (a) antigen solution or immune serum dilution, respectively, and (b) salt solution (controls).* With occasional agitation, these cell suspensions were incubated one hour at 37°C, the cells were then washed twice in saline, and 1% cell suspensions in saline were prepared of both "sensitized" and "non-sensitized" (control) cells.

(4) *Hemolysis titration.* Twofold serial dilutions of antigen solution or of immune serum, respectively, in 0.2 ml amounts, were prepared in duplicate. To both series of tubes were then added 0.2 ml amounts of a 1:10 dilution of complement. One series of tubes received 0.2 ml amounts of 1% sensitized

1. Kondo, S., *Z. Immunitäts.,* 1923, v36, 76.

2. Fisher, S., and Keogh, E. V., *Nature,* 1950, v165, 248.

3. Boivin, A., and Mesrobeanu, L., *C. R. Soc. Biol.,* 1933, v112, 76.

* Cells which were exposed to solutions of bacterial antigens or to serum proteins in the manner just described will be referred to as sensitized cells.

TABLE I.
Effect of Complement Upon Antigen-treated Erythrocytes in the Presence of Non-Hemolytic Corresponding Immune Sera.

Immune serum employed	Designation of erythrocytes used	Reciprocal of final serum dilution									Serum control	Complement control	Cell control
		50	100	200	400	800	1600	3200	6400	12800			
Rabbit anti-*E. coli* serum	A	4	4	4	4	4	3	3	2	0	0	0	0
(Rabbit No. 1)	B	4	4	3	1	0	0	0	0	0	0	0	0
	C	4	4	4	3	1	0	0	0	0	0	0	0
	D	3	2	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0	0	0	0	0
Rabbit anti-*E. coli* serum	A	4	4	4	4	4	3	2	1	0	0	0	0
(Rabbit No. 1)	D	0	0	0	0	0	0	0	0	0	0	0	0
Absorbed with sheep erythrocytes	E	4	4	3	2	1	1	0	0	0	0	0	0
Rabbit anti-*S. typhosa* serum	E	4	4	4	4	3	2	1	0	0	0	0	0
(Rabbit No. 2)	A	3	1	1	0	0	0	0	0	0	0	0	0
	D	0	0	0	0	0	0	0	0	0	0	0	0
Rabbit serum vs. human serum	F	2	4	4	4	4	4	3	1	0	0	0	0
from type O donor	G	0	0	0	0	0	0	0	0	0	0	0	0

A = Sheep erythrocytes, sensitized with *E. coli* extract.
B = Human type O erythrocytes, sensitized with *E. coli* extract.
C = Rabbit erythrocytes, sensitized with *E. coli* extract.
D = Sheep erythrocytes, not sensitized (control cells).
E = Sheep erythrocytes, sensitized with *S. typhosa* extract.
F = Rabbit erythrocytes, sensitized with human plasma from type A donor.
G = Rabbit erythrocytes, not sensitized (control cells).
H = Human type O erythrocytes, not sensitized (control cells).
4 = Complete lysis; 0 = No lysis; 3, 2, 1 = Degrees of incomplete lysis. Results were read after 1 hr incubation at 37°C.

cells, the other series equal amounts of non-sensitized cells. A serum or antigen control tube, in which complement was replaced by an equal volume of saline, a complement control tube, in which serum or antigen was replaced by saline, and a cell control tube containing erythrocytes and saline, were also set up. The total volume in each tube was brought to 1 ml by the addition of saline. The results were read after one hour incubation at 37°C.

Results. (1) A summary of findings pertaining to the lysis of erythrocytes sensitized with different antigens upon exposure to the corresponding immune sera, and complement is presented in Table I. It may be seen that sensitization of red cells was accomplished by human plasma as well as by bacterial extracts. Further, the results show that sensitized cells were lysed even in high dilutions of the immune sera specific for the sensitizing agent (1:400 to 1:12800), and that removal of normal hemagglutinins from such sera failed to depress the hemolytic activities against sensitized cells significantly, while hemolysis of non-sensitized cells was completely abolished. No lysis occurred when either complement or immune serum, or both reagents were omitted. An antigenic relationship between *E. coli*, strain H, and *S. typhosa* O 901, evident from cross-agglutination,† expressed itself in the lytic action of the anti-*S. typhosa* serum on erythrocytes sensitized with *E. coli* extract (1:200), and by the lysis of red cells sensitized with *S. typhosa* extract in the presence of anti-*E. coli* serum (1:1600). Red cells sensitized with the bacterial extracts occasionally were darkened, and tended to clump. To ascertain whether these noticeable changes in the erythrocytes could cause increased sensitivity toward lysis by complement, a 2% suspension of such cells was prepared in saline, and a routine complement titration(4) was carried out, using two units of amboceptor. Both normally appearing, non-sensitized cells, and discolored sensitized cells were equally sensitive to complement. (0.2 ml of a 1:40 dilution of guinea pig serum was the least amount that caused complete lysis in the presence of 2 units of amboceptor).‡

(2) Attempts were made to reverse the process by sensitizing erythrocytes with non-hemolytic immune sera, and to induce lysis by adding such cell suspensions to serial dilutions of the corresponding antigens in the presence of complement. The available rabbit immune sera proved to be unsuitable for the sensitization of sheep cells, since dilutions smaller than 1:10 contained sufficient normal anti-sheep antibody to induce lysis of these cells when complement was added. Recourse was therefore taken to the use of rabbit erythrocytes. Red cells from the defibrinated blood of two rabbits were washed in saline, exposed (in 1% concentration) to a 1:4 dilution of rabbit anti-*E. coli* immune serum for one hour at 37°C, washed once, made up to a 1% suspension in saline, and then added in the usual manner to serial dilutions (from 1:10 to 1:25600) of the *E. coli* extract, in the presence of complement. No lysis ensued. Upon overnight storage of the test in the ice-box, hemagglutination was evident in the tubes containing sensitized cells and extract dilutions from 1:10 to 1:640. Sensitized cells were not agglutinated by the higher extract dilutions, or in the absence of bacterial extract. Non-sensitized cells were not agglutinated.

Since agglutination, but not lysis, had occurred in the previous experiment, an attempt was made to test the efficacy of sheep erythrocyte sensitization with a 1:4 dilution of the *E. coli* antiserum (absorbed with sheep red cells). Aliquots of 0.3 ml of a 1% suspension of sensitized cells, and equal volumes of a similar suspension of non-sensitized erythrocytes, were added to 0.3 ml volumes of serial

†In slide agglutination tests(5), using suspensions of living cells, the anti-*S. typhosa* serum still agglutinated *E. coli* when diluted 1:25, and the anti-*E. coli* serum still agglutinated *S. typhosa* when diluted 1:5.

4. Boyd, W. C., Fundamentals of Immunology, Interscience Publishers, Inc., New York, 1947.

‡ Similarly discolored sheep cells, obtained by treating 5 ml of a 1% suspension in saline with 0.25 ml of a commercial 3% solution of H_2O_2 for 5 minutes at 37°C, proved to be more sensitive to a sample of fresh guinea pig serum which possessed normal sheep cell agglutinins than untreated cells. Untreated cells were lysed in dilutions less than 1:5; while treated cell were lysed even by a 1:20 dilution of the guinea pig serum.

PROTOCOL AND TABLE II.

Fixation of Complement by Sensitized Erythrocytes in the Presence of Corresponding Antigens or Antisera, Respectively.

1	2	3	4	5	6	
Tube	0.2 ml of 1% erythrocyte suspensions	*E. coli* antigen solution (0.2 ml)	*E. coli* antiserum* dilution (0.2 ml)	G. pig complement (1:5 dilution) ml	Saline ml	
A	Sensitized with *E. coli* antiserum*	1:8	—	.2	.4	Incubated for 1 hr in ice bath, then centrifuged and supernate decanted
B	"	1:16	—	.2	.4	
C	"	Saline	—	.2	.4	
D	Sensitized with *E. coli* antigen dil'n	—	1:10	.2	.4	
E	"	—	1:40	.2	.4	
F	"	—	Saline	.2	.4	

7	8	Supernates 9								10	11
		Aliquots of 0.15 ml to 0.50 ml of diluted supernates (increments of 0.05 ml) were added to 0.2 ml fresh 2% sheep erythrocyte suspension + 2 units amboceptor incubated 1 hr at 37°C									
Tube	Saline, ml	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	Saline	Saline, ml		Degree of hemolysis
		Degree of hemolysis											
A	2	0	1	1	3	3	4	4	4	0	1	Incubated	0
B	2	0	1	1	2	3	4	4	4	0	1	1 hr	0
C	2	0	1	2	3	4	4	4	4	0	1	at	0
D	2	0	0	0	0	1	2	2	2	0	1	37°C	4
E	2	0	0	0	1	1	3	3	4	0	1		4
F	2	0	1	1	2	4	4	4	4	0	1		0

Key to symbols — Same as in Table I.

* — The antiserum had previously been absorbed with sheep erythrocytes.

twofold dilutions of a chicken anti-rabbit euglobulin serum (absorbed with sheep erythrocytes). After 1 hour at 37°C, and overnight incubation in the icebox, sensitized erythrocytes were agglutinated by chicken serum dilutions up to 1:160, while non-sensitized erythrocytes were agglutinated in dilutions up to 1:20 only.

In view of this evidence of actual adsorption of rabbit euglobulins onto the surface of sheep erythrocytes, it now appeared desirable to investigate whether at least some of the adsorbed euglobulins were *E. coli* agglutinins. Serial twofold dilutions of the *E. coli* antiserum used in sensitizing sheep erythrocytes, and similar dilutions of a sample of the same serum which had not been employed in the sensitization of the sheep erythrocytes, were tested for *E. coli* agglutinins. The results of the agglutination tests(5) showed that a reduction in *E. coli* agglutination titer from 1:1024 (prior to its use for sheep erythrocyte sensitization) to 1:256 (after sheep cell sensitization) had occurred.

Since erythrocytes sensitized by their exposure to *E. coli* antiserum had apparently absorbed some *E. coli* agglutinins, as shown by the results of the previous experiments and by the agglutination of such cells by *E. coli* antigen, it remained to be seen why lysis of such cells did not take place in the presence of *E. coli* antigen upon addition of complement. In order to determine whether the sensitized erythrocytes combined with complement at all in the presence of *E. coli* antigen, a complement-fixation test was carried out by the method outlined in Protocol and Table II.

It may be seen from column 9 of the table that complement was fixed in appreciable quantities only by those sheep cells which had

5. Edwards, P. R., and Bruner, D. W., Serological Identification of Salmonella Cultures, University of Kentucky Agricultural Experiment Station, Circular 54, 1942.

been sensitized with *E. coli* antigen and which subsequently were exposed to *E. coli* antiserum (tubes D and E). Fixation of complement by these cells during the incubation in the ice bath was followed by their lysis on subsequent exposure to 37°C (Column 11, tubes D and E). Sheep cells which had been sensitized with *E. coli* antiserum failed to fix significant amounts of complement when they were exposed to dilutions of *E. coli* antigen solution (Column 9, tubes A and B). Subsequent incubation of these cells at 37°C failed to result in their lysis. (Column 11, tubes A and B).

Summary. The data presented in this paper confirm and extend the recent observations of Fisher and Keogh(2). Erythrocytes from sheep, rabbit, and man, which have adsorbed extracts from *E. coli* and *Salmonella typhosa,* or human plasma, are lysed in the presence of complement and of corresponding immune sera for the adsorbed antigens. Evidence for the specificity of the reaction has been presented. Neither appreciable complement fixation, nor lysis occurred when erythrocytes first sensitized with non-hemolytic immune sera were then exposed to complement and the corresponding antigens.

Received May 29, 1950. P.S.E.B.M., 1950, v74.

A Direct Relationship between Indoleacetic Acid Effects on Growth and Reducing Sugar in Tobacco Tissue.* (17973)

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Numerous attempts have been made to relate the effects of auxin (indole-3-acetic acid (I.A.A.); naphthalene acetic acid; and related compounds) on growth of plants with specific changes in metabolism and cellular constituents. In the case of toxic effects of 2,4-dichlorophenoxyacetic acid (2,4-D) decreases or temporary rises and subsequent depletion of available carbohydrates have been reported(1-4). An increase in reducing capacity in tissues, measured by staining, has been obtained from treatments of bean stem cultures with 2,4-D(5).

Recently Christiansen and Thimann(6) have demonstrated a decrease in reducing sugar in *Pisum* stem sections and *Avena* coleoptile sections grown for short periods of time in solutions of various compositions with auxin concentrations which promote growth. However, they report a similar decrease in controls without I.A.A. and even greater decreases of sugar from the addition of respiratory inhibitors, which completely stop growth, such as iodoacetate, arsenite, and fluoride. In general, tests on changes in composition associated with auxin treatments which promote growth have been carried out in short time experiments under conditions leading to net decreases in dry weight of the tissues, and where their continued growth was precluded. It was of interest, therefore, to carry out analyses for changes in cell constituents in tissues supplied with I.A.A. in media adequate for continuous growth over periods of several months. The experiments reported here, with tobacco stem segments

* This project is supported in part by the University Committee, on funds from the Wisconsin Alumni Research Foundation, and in part by a grant in aid from the Cancer Society upon recommendation of the Committee on Growth of The National Research Council.

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