Nausea and occasional vomiting occurred in five patients, with daily doses of over 10 g per day. No other reactions were noted, and no effects were observed clinically or biochemically upon the functions of the kidneys, liver, or hemapoietic tissues.

Seven of the 8 patients died after an expected course of the disease; Case 3 is still alive. Necropsy examination in seven cases failed to reveal histologic evidence of toxic effects or other changes attributable to the ingestion of the chemical.

Conclusion. Daily oral doses of 0.3 to 27 g of monobenzyl ether of hydroquinone, for total doses of 2.9 to 1490 g in 21 to 214 days, had no effect upon the growth of malignant melanoma in 8 patients. Ingestion of monobenzyl ether of hydroquinone produced no toxic effects other than transient nausea and vomiting when the daily dose exceeded 10 g.

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Bactericidal Action Mediated by Antibodies Specific for Heterologous Antigens Adsorbed to Bacterial Cells. (19457)

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Many gram negative bacteria are killed, and some are lysed, when they are exposed to complement and homologous antibody. It is generally believed that the antibody which mediates the killing of the bacterial cells by complement is specific for somatic antigens (1-3), though antibody specific for flagellar antigens has been held responsible for bactericidal serum activity against motile strains of *Proteus*(4).

The present communication, based on data obtained in the course of a more detailed investigation of qualitative and quantitative aspects of the bactericidal reaction,* is concerned with the site at which the antigen-antibody reaction responsible for cellular death occurs.

It is known that bacterial cells may adsorb to their surface components of the medium in which they are grown or suspended(5,6). The presence of some of these adsorbed substances can be demonstrated by the observation that such coated bacterial cells are agglutinated by antibody specific for the adsorbed substances. It appeared of particular interest to determine whether, in the presence of complement, reactions between antibody and adsorbed antigen would cause the death of susceptible bacteria. That bactericidal activity might actually occur as the result of such reactions was indicated by the work of Angerer and Hartoch(7) who demonstrated accelerated lysis of *Vibrio* cells which had first been exposed to suboptimal amounts of bactericidal horse serum and then to rabbit anti-horse serum. Bacterial antigens were chosen as the coating agents in this investigation because their use promised to throw some light on the quantitative aspects of the bactericidal reaction and also on the apparent lack of specificity in the bactericidal action of normal sera. These problems will be dealt with in subsequent papers.

Materials and methods. The antigens employed for the coating of bacteria were extracted with trichloroacetic acid as described by Boivin(8). They were precipitated by the addition of alcohol to a final concentration of

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75% by volume, washed repeatedly in 75% alcohol, and finally dried over calcium chloride under aseptic conditions. When required, sufficient amounts of dry antigen were weighed out to yield the desired volume of the 0.1% solution (in physiological saline) to be used in the coating procedure. In order to coat bacterial cells of one species with antigens derived from another species, the growth from three 18-hour slant cultures was harvested and washed twice with saline. The packed cells were then evenly suspended in 5 ml of the sterile 0.1% solution of antigen, and a similar amount of cells was suspended in saline, to serve as control. After 30 minutes of incubation at room temperature, with occasional agitation, both cell suspensions were diluted with saline until they contained approximately 5 x 10^4 viable cells per ml.† To test the *bactericidal activity* of immune sera against coated (test) and uncoated (control) bacteria, the method of Felix and Olitzki(1) was modified so that more nearly quantitative titrations could be obtained. Serial dilutions of inactivated immune serum, in 0.2 ml amounts, were distributed into a series of sterile tubes and, while the tubes were kept in an ice-water bath, the following reagents were added in quick succession: 0.2 ml of the standard inoculum of bacteria (approximately 10^4 viable cells), 10 minimum hemolytic units of complement (contained in 0.1 ml of a suitable dilution of specifically absorbed fresh guinea pig serum), and 0.5 ml of half-strength broth. After mixing of the contents the tubes were incubated at 37°C for 30 minutes, returned to the ice-water bath, and 2 ml of chilled, quarter-strength broth were added to each tube. Finally two 1 ml aliquots from each tube were transferred to Petri plates and mixed with proteose No. 3 agar at 45°C. Colonies present after 18 hours of incubation at 37°C were counted by the microscopic method described by Felix and Olitzki(1). *Controls* for the sterility of the various reagents, for lack of bactericidal activity of either the immune serum or of complement alone, and a control on the bacterial content of the inoculum (immune serum and complement omitted) were included in each test.

Application of Student's "t" test for statistical significance to a large number of data obtained in these and other bactericidal tests demonstrated that a difference of 15% between the number of colonies present on the inoculum control plates and the number of colonies arising from any given experimental mixture of immune serum, bacteria, and complement, was highly significant. Therefore, the titer of a serum was defined as the highest dilution of the serum which under the standard conditions of the experimental procedure caused the death of 15% or more of the inoculum. In order to *free the immune sera* used in these experiments and the guinea pig sera which were employed as complement of undesired antibodies, absorptions were carried out with massive doses of heat-killed, washed cells of the indicated bacterial strains. Absorptions of the guinea pig sera were carried out at 0-4°C, those of the immune sera at 37°C, for one hour, twice in succession with each absorbing organism. The *immune sera* were prepared by immunizing rabbits with 5 to 6 doses of bacteria. The bacterial cultures were obtained from the stock collections of the Department of Bacteriology and Immunology, Washington University, and the Department of Bacteriology, University of Kentucky.

Results. The data presented in Table I show that cells of each of the 3 *Salmonella* species tested (*S. münchen, S. potsdam* and *S. ballerup*) could be rendered sensitive to the bactericidal action of complement in the presence of an anti-*Escherichia coli* immune serum if they were first exposed to a solution of *E. coli* antigens. This bactericidal activity against coated bacteria was present even in high dilutions of the immune serum (1:640 to 1:20,480), while uncoated (control) cells were not killed by any of the serum dilutions tested.

It appeared of interest to determine, in a preliminary manner, whether cells of other gram negative bacterial species could also be conditioned to the killing action of comple-

† Because the serial dilutions required in this step generally involved a 10000-fold dilution of the suspending fluid, it was not necessary to wash the cells free of unadsorbed antigen.

TABLE I. Bactericidal Action Mediated by *Escherichia coli* Antiserum Against *Salmonella* Cells Which Have Been Coated with *E. coli* Antigens.

	Anti-*E. coli* serum absorbed with											
	S. münchen tested against *S. münchen*				*S. potsdam* tested against *S. potsdam*				*S. ballerup* tested against *S. ballerup*			
	Uncoated		Coated*		Uncoated		Coated*		Uncoated		Coated*	
Final dilution of *E. coli* antiserum	No. surv.	% killed	No. surv.	% killed	No. surv.	% killed	No. surv.	% killed	No. surv.	% killed	No. surv.	% killed
1:80	2790	0†	2210	35	2920	0	1750	36	3310	0	2370	34
1:320	2860	0	2290	32	2940	0	1860	32	3240	0	2610	28
1:1280	2750	0	2610	24	2880	0	2470	0	3210	0	3050	16
1:5120	2620	0	2650	22	2830	0	2680	0	3280	0	3230	0
1:20480	2660	0	2930	15			2660	0			3540	0
1:81920	2740	0	3010	0								
Controls												
Serum	2740		3360		2880		2750		3190		3580	
Complement	2780		3450		2880		2720		3220		3610	
Saline	2720		3460		2820		2700		3240		3550	
Titer	<1:80		1:20480		<1:80		1:640		<1:80		1:1280	

* Coated with antigens extracted from *E. coli*. † Less than 15% of inoculum killed.

Complement used was absorbed twice with *E. coli*, and twice with *S. münchen*, *S. potsdam*, or *S. ballerup*, respectively.

TABLE II. Bactericidal Action of Anti-*S. typhosa* O 901 Serum Against Cells of Unrelated Bacterial Species, Coated with *S. typhosa* O 901 Antigens.

	Anti-*S. typhosa* serum absorbed with											
	K. pneumoniae tested against *K. pneumoniae*				*S. marcescens* tested against *S. marcescens*				*P. morgani* tested against *P. morgani*			
	Uncoated		Coated*		Uncoated		Coated*		Uncoated		Coated*	
Final dilution of *S. typhosa* antiserum	No. surv.	% killed	No. surv.	% killed	No. surv.	% killed	No. surv.	% killed	No. surv.	% killed	No. surv.	% killed
1:40	n.t.†		n.t.†		2740	0‡	1750	35	3280	0	1680	27
1:80	3010	0	2950	0	2670	0	2090	22	3210	0	1920	17
1:160	2970	0	3010	0	2710	0	2740	0	3190	0	2310	0
1:640	2990	0	3070	0	2690	0	2670	0	3240	0	2400	0
1:1280	3020	0	3110	0	2630	0	2740	0	3180	0	2360	0
Controls												
Serum	2970		3180		2610		2740		3090		2300	
Complement	3030		3200		2570		2670		3160		2340	
Saline	3000		3160		2690		2630		3180		2370	
Titer	<1:80		<1:80		<1:40		1:80		<1:40		1:80	

* Coated with antigens extracted from *S. typhosa* O 901. † n.t. = not tested.

‡ Less than 15% of inoculum killed.

Complement used was absorbed twice with *S. typhosa* O 901, and twice with *K. pneumoniae*, *S. marcescens*, or *P. morgani*, respectively.

ment and of antibody specific for substances adsorbed to their surface. One strain each of *Klebsiella pneumoniae*, *Serratia marcescens*, and *Proteus morgani* was selected, and cells of these strains were exposed to a solution of antigens derived from *Salmonella typhosa*. The coated cells, as well as uncoated cells of the test strains, were then exposed to serial dilutions of an anti-*S. typhosa* serum which had been previously freed of natural antibodies against the test strains by suitable absorptions. The results, presented in Table II, show that both the *S. marcescens* cells and the *P. morgani* cells which had adsorbed antigens from *S. typhosa* became sensitive to the bactericidal action of complement in the presence of anti-*S. typhosa* serum, while the mucoid strain of *K. pneumoniae* apparently failed to acquire sensitivity to this serum.

Discussion and summary. The data presented support the view that the bactericidal reaction is mediated by the reaction of antibody (and complement) with antigens located at the surface of the bacterial cells. Since it

may be assumed, *a priori*, that sensitization to killing by complement requires attachment of certain minimum amounts of antibody to the cell, and this in turn depends on the presence of a sufficient number of specific antigen sites, the findings reported here may serve to explain the reported successful sensitization of a heavily flagellated *Proteus* strain by anti-flagellar antibody(4), and the consistent failure to observe sensitization of the less heavily flagellated *Salmonella* strains by antibody specific for their flagellar antigens.

The apparent differences in acquired sensitivity of the various test strains to the bactericidal effect of complement and of antibody specific for adsorbed antigens need not be considered significant. In some instances, such as in the case of the mucoid *Klebsiella* strain, resistance may be inherent in the strain (9), in other cases the composition of the cell surface may limit its capacity to adsorb the particular test antigen. Optimum conditions for adsorption were not extensively investigated.

It is believed that findings presented here should be taken into consideration in the interpretation of apparently non-specific bactericidal activities of sera. Furthermore, since the bactericidal test is an extremely sensitive method for the detection of small amounts of antibody, further refinement of the technic may lead to the development of methods suitable for the detection of trace amounts of antibody against antigens that may be adsorbed to suitable bacterial strains.

Conclusion. The bactericidal activity of complement is mediated by the reaction between antibody and antigens located at the surface of bacterial cells. These surface antigens need not be constituents of the cell but may be substances which have been artificially adsorbed to its surface.

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Effects of Exposure to Cold and of Dietary Restriction upon Globulin Nephritis in Rabbits.* (19458)

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Glomerulonephritis has been induced in both rats and rabbits by a variety of experimental procedures(1-9). These have included intravenous injection of heterologous serum proteins(1-4), anti-kidney antibodies(5-7), and extracts of ground kidney and streptococci(8,9). Most attempts to increase or decrease the severity of experimental nephritis have been made with so-called "nephrotoxic" nephritis, *i.e.*, that produced by injection of an anti-kidney serum from another species. This type of nephritis has been inhibited in rabbits by preliminary general exposure of the body to x-radiation(10). In rats nephrotoxic nephritis has been prevented by restriction of dietary protein(11).

A very severe acute glomerulonephritis has been induced in rabbits by massive intravenous injections of bovine serum gamma globulin(12-14). The present report deals with the

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