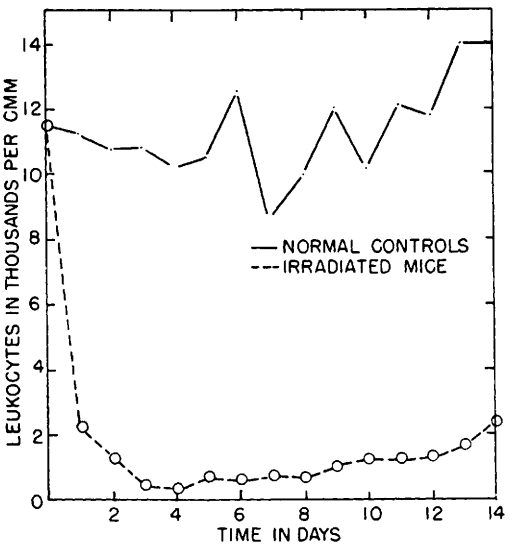

FIG. 1. Effect of gamma radiation 680 (rep.) upon level of leukocytes in peripheral blood of mice.

loss to the cellular defensive mechanism of an irradiated animal. It is indicated from these data that granular leukocytes and the lymphoid-macrophage system may be of greater importance in immunity to Trichinella infections than has formerly been considered.

Summary. 1. Cobalt^{60} gamma whole body irradiation of 550 to 650 (rep) increased the susceptibility of Swiss mice to infections with *Trichinella spiralis*. 2. Exposure of Trichinella-immune mice to 600 (rep) before administering a challenge infection destroyed their immunity to reinfection. 3. Data are presented on the effects of 680 (rep) radiation upon the total leukocyte count in the peripheral blood of mice. The sharp reduction in the number of circulating leukocytes may indicate that the role of cellular immunity should be emphasized more in acquired immunity to infections with *Trichinella spiralis*.

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Immunization of Rabbits with Type II Pneumococcal Polysaccharide.* (19675)

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The specific soluble substances of the pneumococci have been reported to be antigenic in man, mouse, horse, dog and cat(1), but all attempts to immunize rabbits with the type-specific polysaccharides have failed. For example, Avery and Morgan(2), Schiemann(3), and Avery and Goebel(4) reported attempts to immunize rabbits with pneumococcal polysaccharides, but they failed to detect type-specific precipitins, agglutinins, or protective antibodies in the sera of the treated rabbits. Schiemann(3) employed 1.0 to 1.5 mg of type II polysaccharide; Avery and Goebel(4) used as much as 18 mg and as little as 180 γ of type I pneumococcal polysaccharide in experiments to immunize rabbits. Although they failed to find type-specific antibodies, Avery and Goebel(4) were able to detect type I polysaccharide in the sera of their treated rabbits. It appeared likely that these investigators had employed "paralyzing" doses of the

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type-specific polysaccharides. This "immunological paralysis" with large doses of polysaccharide had been noted in mice(5). The purpose of this paper is to present evidence that small quantities of type II pneumococcal polysaccharide injected intravenously or intradermally are antigenic in rabbits.

Materials and methods. Type II pneumococcal polysaccharide, Lot 31T,‡ employed in this investigation contained 0.42% nitrogen and gave a positive test with Folin-Ciocalteau reagent(6,7). The viscosity of a 0.1% solution of the polysaccharide in 0.9% sodium chloride was 1.55 at 20.0°C. One-tenth gamma of the polysaccharide actively immunized mice against 10,000 LD of pneumococcus type II.

In one experiment each of 6 New Zealand white rabbits from 1700-2270 g in weight received intracutaneously on the right flank at weekly intervals 10 γ of pneumococcal type II polysaccharide in 1.0 ml of 0.85% NaCl. During the course of immunization each rabbit received 40 γ of type-specific polysaccharide.

In another experiment each of 6 New Zealand white rabbits weighing 1800-2500 g was inoculated intravenously with a saline solution containing 5 γ of pneumococcal type II polysaccharide per ml. Each rabbit received 14 injections of 1 ml amounts over a period of 4 weeks or a total of 70 γ of polysaccharide.

A sample of serum for control purposes was obtained from each rabbit before the start of the immunization schedules and a second sample was secured on the 7th or 8th day following the final injection of polysaccharide to test for specific antibodies. The sera from the animals in the same test group were pooled for storage in sealed ampules at —20°C.

The rabbits which had been bled for test sera were tested by the method of Goodner(8) for active immunity against pneumococcus type II. The test and control animals were challenged on the left flanks with 0.2 ml doses of varying dilutions of a 14-hour culture of pneumococcus type II grown in yeast-tryptose-phosphate broth containing 5% fresh rabbit blood. Rabbits failing to develop a fever, or edematous lesions and showing no loss of appetite or weight were considered to exhibit immunity(8).

Mouse-protection tests for assessing each pool of rabbit antiserum were performed by employing male or female Swiss mice, strain CFW. Each mouse received by intraperitoneal injection a mixture of 0.2 ml of serum and 0.3 ml saline several seconds before the administration by the same route of 0.5 ml of a diluted 15-16 hour culture of pneumococcus type I or II grown in blood broth. Groups of three 18-22 g mice were used for each dilution of bacterial suspension. The mice that survived for at least 96 hours were considered passively immune. The Quellung reaction, using type-specific antiserum and the peritoneal exudate of mice dead within 96 hours, was employed to determine the presence of pneumococcus type II.

Blood-broth cultures of pneumococcus type I or II employed to challenge the rabbits and mice were obtained by seeding blood-broth with the heart's blood of mice injected intraperitoneally with the fresh broth cultures of pneumococci. In the case of the 14-hour cultures of pneumococcus type II used to challenge the rabbits, 10-fold dilutions were made from equal volumes of yeast-tryptose-phosphate broth and saline. With the 15-16 hour cultures of pneumococci employed in the mouse-protection tests a 5-fold dilution was made first and then successive 10-fold dilutions.

Results. Table I shows that all 12 rabbits injected either intradermally or intravenously with pneumococcal type II polysaccharide exhibited signs of active immunity against an intradermal infection with type II pneumococcus. The test animals failed to develop fever and 5 had slight transitory lesions. All control rabbits challenged with dilutions of 10^{-1} and 10^{-2}, two at 10^{-3}, and two at 10^{-4} developed fever and edematous lesions.

Table II presents the findings of mouse-protection tests that employed the pooled serum from the 6 rabbits which had been immunized intradermally with type II pneumo-

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TABLE I. Active Immunization of Rabbits by Intradermal or Intravenous Injection of Type II Pneumococcal Polysaccharide

History of rabbits	Challenge with dilutions of type II pneumococcus* 10^{-1}	10^{-2}	10^{-3}	10^{-4}
Received intradermally type II polysaccharide	3/3†	3/3	——	——
Received intravenously type II polysaccharide	3/3	3/3	——	——
Normal controls	0/3	0/3	1/3	1/3

* The challenge dose was 0.2 ml of a diluted 14 hr blood broth culture given intradermally.
† Numerator = No. of rabbits exhibiting active immunity; denominator = No. of rabbits challenged.

TABLE II. Demonstration of Type-Specific Protective Antibodies for Mice in Serum of Rabbits Injected Intradermally with Pneumococcal Type II Polysaccharide.

Homologous challenge

Mice	Dilutions of homologous challenging organism† 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Injected with immune serum	0/3*	0/3	1/3	3/3	3/3	3/3	3/3	——	——
Injected with normal serum	0/3	0/3	0/3	0/3	0/3	0/3	0/3	——	——
Normal controls	——	——	0/3	0/3	0/3	0/3	0/3	0/3	2/3

Heterologous challenge

Mice	Dilutions of heterologous challenging organism‡ 10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Injected with immune serum	0/3*	0/3	0/3	0/3	0/3	0/3	0/3	——
Injected with normal serum	0/3	0/3	0/3	0/3	0/3	1/3	1/3	——
Normal controls	——	0/3	0/3	0/3	0/3	1/3	1/3	3/3

* Numerator = No. of mice surviving at least 96 hr; denominator = No. of mice challenged.
† 0.5 ml of diluted 15 hr blood-broth culture of pneumococcus type II.
‡ 0.5 ml of diluted 15 hr blood-broth culture of pneumococcus type I.

TABLE III. Demonstration of Protective Antibodies for Mice in Serum of Rabbits Injected Intravenously with Pneumococcal Type II Polysaccharide.

Mice	Dilutions of homologous challenging organism† 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Injected with immune serum	0/3*	0/3	0/3	3/3	3/3	3/3	3/3	——	——
Injected with normal serum	0/3	0/3	0/3	0/3	0/3	0/3	0/3	——	——
Normal controls	——	——	0/3	0/3	0/3	0/3	0/3	1/3	3/3

* Numerator = No. of mice surviving at least 96 hr; denominator = No. of mice challenged.
† 0.5 ml of diluted 16 hr blood-broth culture of pneumococcus type II.

coccal polysaccharide. It can be seen that 0.2 ml of the pooled immune serum protected mice against at least 10,000 LD of pneumococcus type II, but probably not against a single LD of pneumococcus type I. The pooled normal serum of the same 6 rabbits is seen to have been ineffectual in protecting mice against either pneumococcus type II or type I.

The data given in Table III show that the pooled serum of rabbits which had been immunized intravenously with type II pneumococcal polysaccharide protected the mice against almost 10,000 LD of type II pneumococcus, whereas the pooled normal serum of the same animals failed to protect against the homologous organism.

Neither of the 2 pools of immune serum gave a precipitin test with the type II pneumococcal polysaccharide, nor they did agglutinate a suspension of type II pneumococcus killed with heat and formalin.

Discussion. The results of these experiments afford evidence that the pneumococcal type II polysaccharide in minute amounts is

antigenic in rabbits and suggest that other pneumococcal type-specific carbohydrates in extremely small quantities would also elicit the formation of mouse-protective substances in rabbits. In view of Felton's work(5) employing mice it is possible that the immune mechanism in rabbits in response to minute amounts of type-specific polysaccharide and perhaps even to heat-killed pneumococci could be "paralyzed" with large quantities of the same specific soluble substance.

Dubos(9) showed that heat-killed pneumococcus type I treated with bacteriolytic enzymes showed a loss of antigenicity in rabbits; these findings were based on the failure to detect type-specific precipitins in the sera of his experimental animals. It is possible that the sera of these rabbits contained mouse-protective antibodies.

Dubos and MacLeod(10) found that leucocytes contained an enzyme which inactivated the capsular antigen of pneumococcus type I *in vitro*. They suggested that this same enzymatic activity may take place in the skin of rabbits and thus account for the lack of type-specific antigenic response to the intradermal injection of heat-killed pneumococci. However, since the authors reported the failure of the enzyme to destroy the type-specific polysaccharide, one might predict from our findings that the intradermal injection of heat-killed pneumococci into rabbits would result in the production of type-specific mouse-protective antibodies.

Angevine(11) in studies on the antigenic response of rabbits to the intracutaneous injection of heat-killed pneumococcus type I reported the absence of type-specific agglutinins in the sera of the treated rabbits 33 days after the initial inoculation, but on continued intracutaneous injections, type-specific agglutinins were demonstrable 63 days after the first injection. If mouse protection tests had been performed, it is possible that mouse-protective substances would have been detected in the sera of the treated rabbits showing no agglutinin content after 33 days.

From our work one may postulate that the mouse-protective antibody elicited in rabbits by the type II pneumococcal polysaccharide is the type-specific anti-carbohydrate present in extremely small amounts. It was thought at first that this mouse-protective substance might be a univalent or so-called incomplete antibody. However, we were unable to show in a serum known to contain type II mouse-protective substances the presence of univalent antibodies employing the quantitative technic of Heidelberger *et al.*(12). Furthermore, precipitins were not demonstrable with the quantitative micro-technic described by Heidelberger and MacPherson(6,7). The failure to find in the mouse-protective serum univalent or precipitating antibodies employing quantitative technics is in accord with the findings of Stillman and Goodner(13). These authors suggested that the mouse-protective test was much more sensitive than the agglutination test. Of great importance, to an understanding of these results, Northrop and Goebel(14) showed that 0.5 γ of dissociated pneumococcal type I antibody N was capable of protecting mice against 1,000,000 LD of pneumococcus type I, whereas 16 γ were necessary to agglutinate heat-killed pneumococcal type I cells. Consequently an immune serum protective against 10,000 LD of pneumococcus type II would contain insufficient antipolysaccharide to be detected with the micro-technics employed by Heidelberger, *et al.* (6,7,11).

Summary. 1. Type II pneumococcal polysaccharide was found to be antigenic in rabbits. 2. Rabbits injected intradermally or intravenously with minute amounts of type II pneumococcal polysaccharide developed an active resistance to intradermal infection with type II pneumococcus. 3. The pooled serum of rabbits injected intradermally with the polysaccharide protected mice against 10,000 LD of pneumococcus type II, but not against pneumococcus type I. 4. The pooled serum of rabbits injected intravenously with the polysaccharide protected mice against almost 10,000 LD of pneumococcus type II. 5. It is suggested that previous failure to immunize rabbits with type-specific polysaccharides may be attributed to the use of excess polysaccharide resulting in "immunological paralysis." 6. The evidence shows that the mouse-protective antibody is the specific antipolysaccharide which occurs in amounts too small to be

detected by quantitative precipitin technics. 7. An attempt to demonstrate univalency of the type-specific mouse-protective antibody failed.

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Development of Resistance to 4-Amino-N^{10}-Methyl Pteroylglutamic Acid (Amethopterin) by *Leuconostoc citrovorum*.* (19676)

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In children with acute leukemia, whose disease initially responds well to treatment with 4-amino-N^{10}-methyl pteroylglutamic acid (Amethopterin), there develops in the leukemic process, after 6 to 18 months, refractoriness to therapy(1-4). The elucidation of the mechanisms through which this resistance is established would be of extreme importance to the practical chemotherapy of this disease, and hence have been studied in several different systems. The induction of resistance to amethopterin in 2 strains of mouse leukemia has been demonstrated(5,6). The development has also been reported of an amethopterin-fast strain of *Streptococcus faecalis* which, in the absence of amethopterin, maintained its quantitative requirement of pteroylglutamic acid (PGA), but was capable of establishing maximum growth in media containing amethopterin, 4-amino PGA (aminopterin), and 4-amino pteroyl aspartic acid (amino-an-fol) in the absence of PGA(7,8). Studies with this resistant *S. faecalis*(9) have given some insight into the mechanism of the resistance, as well as the role of PGA and citrovorum factor (CF) in the process of cell metabolism.

Since CF has been shown to be a more direct competitor of aminopterin and amethopterin in *S. faecalis*(10), mouse leukemia (11), and human leukemia(12), it was thought again that a biological system specifically requiring CF might be of value for further study of amethopterin resistance.

Method. *Leuconostoc citrovorum* (ATCC No. 8081) was grown for 18 hours on the CF assay medium of Sauberlich and Baumann (13) in the presence of 1 mγ/ml of leucovorin (synthetic CF Lederle),‡ the culture was centrifuged and washed once with saline and inoculated into the same medium with amounts of amethopterin ranging from 0-500 mγ/ml and incubated at 37°C. In 24 hours there was half maximum growth at 50 mγ/ml

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