Hp. Adrenal glands, therefore, are not essential for the development of this response. Levels achieved in the adrenalectomized rats, however, were significantly lower ($p<0.01$) than those of the control group. It is of interest that similar findings have been reported in studies of the response of fibrinogen levels to trauma in adrenalectomized rats(5).

When replacement steroid therapy was undertaken, a dissociation of corticoid effects was seen. Adrenalectomized animals on corticoids received full protection from the lethal effects of the turpentine abscess, but their Hp responses were not affected. That the doses of corticoids used were adequate is demonstrated by the fact that all treated rats survived this rather intense stress. It may be speculated that higher doses of steroids might have induced a normal Hp response. The intact animal subjected to inflammatory stress probably increases his steroid output above the basal levels necessary for maintenance of life, and it is impossible to say whether the replacement doses used in these experiments were fully equivalent to the endogenous secretion of corticoids among the animals with intact adrenals. However, the fact that Hp values among the steroid-treated rats were *no* higher than those among the untreated adrenalectomized rats suggests that the replacement dose was not the determining factor. If it were, one would expect at least a value intermediate between that of the adrenalectomized group and the normal group, with steroid doses high enough to assure 100% survival. It is concluded that the role of the adrenals is permissive; in their absence an Hp response to tissue injury occurs but is significantly impaired.

Summary. The response of serum haptoglobin to turpentine-induced inflammation in intact and adrenalectomized rats was studied. Adrenalectomized rats maintained on saline developed a rise in serum Hp, but one which was significantly less than that of the control animals. Replacement corticoid therapy in adrenalectomized rats improved survival markedly, but failed to restore the response of serum Hp to normal.

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Antibodies to Rabbit γ-Globulin After Immunizing with Various Preparations of Autologous γ-Globulin.* (28104)

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Current evidence indicates that circulating autologous protein injected into animals does not produce antibody unless it has first been treated by methods which produce some degree of denaturation or alteration, however slight. Ample proof of the antigenicity of slightly altered autologous γ-globulin has been presented by Milgrom and Witebsky (1) and by McCluskey, Miller and Benacer-

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raf(2), who demonstrated that rabbits injected with frozen and thawed or otherwise denatured autologous γ-globulins developed antibody reacting primarily with human γ-globulin. Perhaps closer to physiological conditions would be the demonstration of the antigenicity of autologous antigen-antibody complexes or immune precipitates. Since much is already known concerning the antigenic behavior of human and rabbit γ-globulin subjected to enzymatic splitting(3-5), it seemed appropriate to determine whether autologous split protein could function as antigen capable of producing antibody to buried determinants not present in the native uncleaved molecule. Experiments were designed to determine: 1) if immunization alone with antigen and later with autologous antigen-antibody complexes would produce detectable auto-antibody; and 2) if immunization with autologous enzyme-split protein could produce auto-antibodies to hidden determinants or to unsplit autologous protein.

Methods and materials. Immunization procedures. Six rabbits (562, 564, 565, 566, 568 and 569) were hyperimmunized over a 10-week period with horse spleen ferritin (Pentex. Lot F76), receiving 0.5 mg of ferritin with complete Freund's adjuvant each week. The horse ferritin was homogeneous on electrophoresis and contained no detectable equine γ-globulin when tested in Ouchterlony plates or by immunoelectrophoresis against rabbit anti-horse serum antiserum. Six weeks after the initial hyperimmunization, quantitative precipitin curves were done on the rabbit antisera and 3 rabbits (562, 566, and 568) were then given 3 weekly intraperitoneal injections of their autologous immune precipitates made at equivalence. Two rabbits (564 and 565) received their soluble autologous immune complexes made by adding ferritin to autologous antibody in moderate antigen excess. Both immune precipitates and complexes were injected with an equal volume of complete Freund's adjuvant. Seven months after these intraperitoneal complex or precipitate injections, one cc of complete Freund's adjuvant alone was given intraperitoneally to 3 rabbits (562, 565, and 568).

Six animals (627, 628, 631, 632, 676 and 678) were injected with autologous γ-globulin prepared by starch block electrophoresis and subsequent papain digestion for 4 hours at pH 7.4, using 1 mg of papain/100 mg of rabbit γ-globulin in the presence of 0.01M cysteine and .002M EDTA. After digestion and dialysis against buffered saline, pH 7.2 Γ/2 0.1, autologous γ-globulin digests were kept at 4°C and injected once a week for 5 weeks together with an equal volume of complete Freund's adjuvant.

Four rabbits (657, 658, 659 and 660) received 5 weekly injections of 5 mg of unaltered autologous γ-globulin separated by zone electrophoresis and kept at 4°C. Seven additional rabbits (511, 512, 513, 514, 515, 379 and 403) were injected once a week for 5 weeks with 5 mg of autologous γ-globulin separated by ammonium sulfate precipitation(1) and frozen and thawed before injection. In all of the rabbits immunized with autologous γ-globulin, an equal volume of complete Freund's adjuvant was used.

Four rabbits (669, 670, 673 and 675) received one cc of complete Freund's adjuvant subcutaneously once a week for 6 weeks.

Tests employed for detection of antibody. Double diffusion in agar(6) and immunoelectrophoresis(7,8) were used for detection of precipitins. Agglutination tests for antibody to human γ-globulin utilized O Rh positive cells coated with 5 different incomplete anti-Rh antibodies. These human incomplete anti-Rh coats varied from those with wide reactivity with anti-γ-globulin antibodies such as the Ripley coat to those having a more limited reactivity (coats Gar., Feimes, 72604, and 2388). Tests for antibody to rabbit γ-globulin included the sensitized sheep cell test using 1/5 basic agglutinating titer of a pooled amboceptor made from antisera obtained from 85 rabbits (Cappel Laboratories Lot 203911) and also using 4 individual rabbit amboceptors prepared from single rabbits selected from different litters. A second test for antibody to rabbit γ-globulin utilized rabbit G-positive cells sensitized by subagglutinating doses of 5 different rabbit incomplete anti-G antisera(9,10) made in individual rabbits (coats Kell., G-13, 702, 703

and 671).† These anti-G antisera contained high proportions of incomplete antibody and, in the sensitizing dilutions used, were not agglutinated by 10 normal rabbit sera.

Density gradient ultracentrifugation employed a 10-40% sucrose density gradient (11,12) in overnight runs of 16 hours.

Coombs' tests were done with the immunized rabbits' own cells washed 6 times before application of antiglobulin serum. Indirect Coombs' tests employed washed normal rabbit cells incubated with doubling dilutions of inactivated rabbit antiserum before washing and application of antiglobulin serum.

Rabbit sera tested with incomplete human anti-Rh antibody coated cells or with the incomplete rabbit antibody were treated at 56°C for half an hour and absorbed with ¼ volume of washed sheep cells. To rule out the participation of immunoconglutinin(13) in these tests, human incomplete anti-Rh antibody coats were also tested after inactivation of the incomplete antibody at 56°C for half an hour.

When papain-split materials were to be tested in agglutination inhibition experiments or in agar diffusion against antisera from rabbits injected with autologous papain-split γ-globulin, prior absorption with papain at equivalence was performed. Such papain-absorbed antisera gave no lines in Ouchterlony plates run with several dilutions of papain.

Inhibition studies. Inhibition tests of anti-human and anti-rabbit γ-globulin antibodies utilized 0.05 ml of inhibiting agent (at concentrations of 1.0, 0.1, and .01 mg/cc) to each 0.5 cc of doubling dilutions of antiserum; after incubation at 37°C for 20 min, the specific indicator system of antibody-coated cells was added. When pure F or S fragments of γ-globulin were used, whole human papain-split γ-globulin was applied to a starch block and the respective isolated fragments separated by electrophoresis. When individual rabbit γ-globulins were used as inhibiting agents, these γ-globulins were prepared by ammonium sulfate precipitation and after washing and dialysis, adjusted to concentrations of 1 mg/cc.

Results. Animals injected with horse spleen ferritin and antigen-antibody complexes. All 6 rabbits hyperimmunized with horse ferritin developed substantial levels of precipitating antibody to ferritin. At the end of prolonged immunization with ferritin alone, 5 out of 6 rabbits showed positive sensitized sheep cell tests using pooled rabbit antibody as amboceptor and all showed positive agglutinations in the incomplete rabbit anti-G system. Three of 6 rabbits showed agglutinating antibody which reacted with human incomplete anti-Rh antibody. These anti-γ-globulin antibodies declined rapidly to low titer or zero during the 6 weeks after immunization.

After 3 injections of autologous immune precipitates, antibody to human γ-globulin, as tested with incomplete human anti-Rh coated cells, rose from 0 to 1:1280 in 2 of the 3 animals. Titers in the sensitized sheep cell test, using pooled hemolysin, and in the rabbit incomplete antibody system showed a rapid similar rise after immune precipitates. The 2 rabbits, receiving 3 intraperitoneal injections of soluble autologous antigen-antibody complexes, also showed striking rises in titer (Table I, rabbit 565) in anti-γ-globulin antibodies reacting with human incomplete antibody, rabbit antibody sensitized sheep cells, and the rabbit incomplete antibody system. Later single intraperitoneal boosters with immune complexes and adjuvant again showed striking rises in titer of both anti-rabbit and anti-human γ-globulin antibodies, whereas single subcutaneous boosters of 1 mg of ferritin and adjuvant as well as single intraperitoneal injections of Freund's adjuvant produced no significant changes in these antibodies.

When the sera of individual rabbits which had received autologous precipitates or complexes were tested with different individual rabbit incomplete antibody coats, marked variation was noted in their specificity for these coats (Table II).

Animals immunized with ferritin and later with autologous complexes showed strongly

† We are indebted to Dr. Aaron Kellner, Dr. Helen Ranney, and Dr. Charles Christian for supplying several of these individual coats.

TABLE I. Serological Data Obtained from Rabbits Immunized with Ferritin and Later Autologous Immune Complexes.

		Normal bleeding 5-11-61	After prolonged immunization with ferritin 7-3-61	2 mo after immunization 9-13-61	After autologous immune complexes* 11-17-61
562	Human incomplete anti-Rh	0	320	0	1280
	Sensitized sheep cell test	0	160	40	320
	Rabbit incomplete anti-G	0	320	0	1280
565	Human incomplete anti-Rh	10	160	160	640
	Sensitized sheep cell test	0	80	0	640
	Rabbit incomplete anti-G	0	160	0	2560

* Rabbit 562 received autologous immune precipitates after immunization with ferritin. Rabbit 565 received soluble autologous immune complexes after initial immunization with ferritin.

positive direct and indirect Coombs' tests.

Animals receiving autologous papain-split γ-globulin. After 5 weeks, 4 of 6 rabbits injected with their own papain-split γ-globulin developed marked anti-γ-globulin antibodies reacting with human incomplete antibody and 5 of the 6 rabbits showed reaction with rabbit antibody sensitized sheep cells, using pooled hemolysin with titers from 1:80 to 1:20,480 (Table II). When the individual rabbit amboceptors were used in the sensitized sheep cell test and individual antisera tested, titers of 1:20 or zero were found with some amboceptors, but titers of 1:160 or 1:320 with others. Anti-rabbit γ-globulin titers, using the rabbit incomplete antibody system, varied between zero and 1:640 in the entire group of 6 rabbits when one rabbit incomplete antibody coat (Kell.) was used. Marked variation in titers with individual rabbit incomplete antibody coats was noted; all 6 of the rabbits immunized with autologous papain-split γ-globulin showed positive agglutinations with at least 4 rabbit incomplete antibody coats. Definite specificity for individual coats was noted, as rabbit 631 showed no agglutination with coat Kell., but a titer of 1:1280 with coat 702; conversely, rabbit 676 showed a titer of 1:640 with coat Kell., but had a titer of 1:20 with coat 702 (Table II). These differences with individual coats were also noted with the other two coats (703 and 671) not listed in Table II.

Strongly positive direct and indirect Coombs' tests were noted in 4 of 6 animals.

Animals receiving autologous γ-globulin alone. Four animals injected with autologous γ-globulin isolated by zone electro-

TABLE II. Serological Data in Rabbits Immunized with: (1) Autologous Immune Complexes and (2) Autologous Papain-Split γ-Globulin.

	Human incomplete anti-Rh titer		Sensitized sheep cell titer—amboceptor #203911		Rabbit incomplete antibody titer					
					Coat Kell.		Coat G-13		Coat 702	
Rabbit	Normal	Post-immun.	Normal	Post-immun.	Normal	Post-immun.	Normal	Post-immun.	Normal	Post-immun.
					Immune complexes					
562	0	1280	0	320	0	1280	0	1280	0	1280
564	0	160	0	640	0	20	0	640	0	10
565	10	640	0	640	0	2560	0	80	0	2560
566	0	40	0	640	0	1280	0	40	0	0
568	0	1280	0	320	0	1280	0	2560	0	1280
					Papain-split γ-globulin					
627	0	1280	0	640	0	640	0	20	0	640
628	0	5120	0	20,480	0	640	0	2560	0	1280
631	0	0	0	0	0	0	0	20	0	1280
632	0	20,480	0	20,480	0	640	0	2560	0	160
676	0	5120	0	640	0	640	0	320	0	20
678	0	10	0	80	0	320	0	20	0	40

TABLE III. Serological Data Obtained in Rabbits Immunized with Autologous γ-Globulins and in Rabbits Given Freund's Adjuvant Alone.

Rabbit	Human incomplete anti-Rh titer		Sensitized sheep cell titer—amboceptor #203911		Rabbit incomplete antibody titer					
					Coat Kell.		Coat G-13		Coat 702	
	Normal	Post-immun.	Normal	Post-immun.	Normal	Post-immun.	Normal	Post-immun.	Normal	Post-immun.
					Autologous γ-globulin					
657	0	0	0	0	0	0	0	160	0	40
658	0	5120	0	0	0	0	0	320	0	160
659	0	320	0	0	0	40	0	320	0	0
660	0	320	0	0	0	40	0	0	0	20
511	0	10,240	0	0	0	0	0	0	0	0
512	0	640	0	0	0	20	0	0	0	0
513	0	10,240	0	10	0	20	0	80	0	0
514	0	40,960	0	0	0	160	0	0	0	0
515	0	163,840	0	20	0	320	0	40	0	0
379	0	10,240	0	0	0	80	0	0	0	0
403	0	5120	0	160	0	160	0	40	0	0
					Freund's adjuvant					
669	0	0	0	0	0	0	0	0	0	0
670	20	40	0	0	0	0	0	0	0	0
673	0	0	0	0	0	0	0	0	0	0
675	0	0	0	0	0	0	0	0	0	0

phoresis and kept at 4°C, and 7 animals immunized with frozen and thawed autologous γ-globulin, showed similar antibodies, except that the latter group showed higher titers against human incomplete anti-Rh antibody. Ten of the 11 rabbits gave titers between 1:320 and 1:163,840 with human incomplete antibody (Table III). Three of the 11 rabbits showed titers of 1:10 to 1:160 with pooled amboceptor in the sensitized sheep cell test. When these 11 antisera were tested with the individual rabbit amboceptors, weakly positive reactions varying between 1:10 and 1:160 were noted with at least one individual amboceptor. Likewise, antibody reacting with rabbit γ-globulin was detected in 10 of the 11 rabbits, using the rabbit incomplete antibody system and the 5 individual coats. Again individual antisera showed apparent preferential reactions with individual rabbit incomplete antibody coats: rabbit 658 showed a titer of 1:320 with coat G-13, but was negative with coat Kell.; in contrast, rabbit 514 showed a negative reaction with coat G-13, but a titer of 1:160 with coat Kell. (Table III).

Animals immunized with Freund's adjuvant alone. One of the 4 animals injected with Freund's adjuvant alone showed a weak but definite reaction with human anti-Rh antibody (1:20) before immunization. After 6 weeks of Freund's adjuvant this titer was 1:40. No reactions were noted in the systems utilizing rabbit antibody-coated sheep cells or the individual rabbit incomplete antibodies either before or after immunization in this group of rabbits.

Physical studies. Density gradient analysis with antisera from both ferritin and autologous split γ-globulin injected rabbits showed anti-human γ-globulin reactivity in both 7S and 19S regions; however, most of the agglutinating activity in sera analyzed was 7S. Similar density gradient experiments with antisera from animals receiving both ferritin and autologous papain-split γ-globulin showed anti-rabbit γ-globulin activity as measured by anti-G rabbit antibody and sensitized sheep cell tests to be 7S and 19S. Different animals showed considerable variation in the ratio of the two classes of antibody.

Inhibition studies. In the ferritin, autologous papain-split γ, and autologous γ-globulin groups, marked inhibtion of anti-human γ-globulin was noted with human Fr II, and moderate inhibition was noted with human γ-globulin split with papain or trypsin. No inhibition was noted with whole rabbit Fr II, but slight inhibition was present with papain-

split rabbit γ-globulin. In the incomplete rabbit antibody system, strong inhibition was noted both by rabbit Fr II and rabbit papain-split γ-globulin. When individual rabbit γ-globulins prepared by ammonuim sulfate precipitation were used in inhibition experiments with rabbit incomplete antibody, distinct inhibition was noted with some rabbit γ-globulins, but none by others. No inhibition was present, using whole human Fr II, but definite inhibition was present, using papain-split human γ-globulin. Similar inhibition of Coombs' tests from ferritin and autologous-split γ-globulin injected rabbits showed marked inhibition by rabbit Fr II and rabbit papain-split γ-globulin. No inhibiton was noted with human Fr II, but human papain-split γ-globulin produced moderate inhibition.

In the anti-human γ-globulin systems tested, pure human S and F fragments from papain digests were tested for inhibiting capacity. The F fragment was the more potent inhibitor for some sera, but both S and F fragments were of similar potency in others. Clear evidence was obtained that the anti-human γ-globulin antibodies reacted with both the S and F fragments of human γ-globulin.

Analysis of antisera by double diffusion in agar gel. Antisera from the ferritin group showed no precipitin lines with either whole or papain-split rabbit γ-globulin though weak lines were noted against whole or split human γ-globulin. Precipitins to rabbit γ-globulin were noted, however, when some antisera to autologous γ-globulin-whole or papain-split reacted with autologous or heterologous papain-split γ-globulin, as well as with human γ-globulin, whole or split (Fig. 1). No precipitins were noted with autologous unsplit γ-globulin or with pooled rabbit Fr II; however, faint precipitins were noted with individual rabbit γ-globulins some of which corresponded to the individual rabbit γ-globulins found to inhibit specific rabbit incomplete antibodies in the rabbit anti-G system. In general antisera reacting with papain-split rabbit γ-globulins or with individual rabbit γ-globulins gave much weaker precipitins in Ouchterlony experiments than with human Fr II.

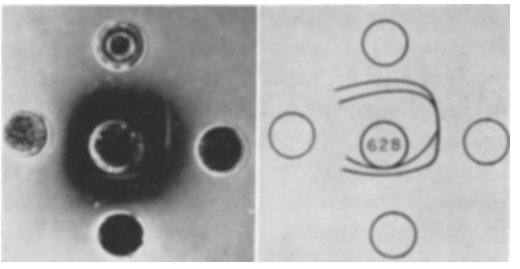

FIG. 1. Photograph and drawing of Ouchterlony plate showing reaction between antiserum made against autologous papain-split γ-globulin (center well 628) and various split γ-globulins. Top and two side wells contain 3 different preparations of papain-split rabbit γ-globulin at 2.0 mg/cc. Bottom well contains papain-split human γ-globulin 0.1 mg/cc.

Discussion. Anti-γ-globulin antibodies reacting with both human and rabbit γ-globulin were produced in rabbits immunized with horse spleen ferritin alone. Titers of such factors could be markedly boosted by restimulation with autologous immune complexes, but not readily by single boosters of ferritin alone nor by intraperitoneal Freund's adjuvants. These antibodies, occurring after immunization with an antigen unrelated to γ-globulin, are similar to the 19S rheumatoid-like factors produced by Abruzzo and Christian(14) in rabbits immunized with coliform organisms over prolonged periods, except that some active 7S agglutinators for both human and rabbit antibody systems were noted among our animals. Also, Aho and Wager (15) were able to produce slight reactivity in the rabbit antibody sensitized sheep cell test after intravenous egg albumin immunization of rabbits. It would appear that immunization with foreign antigen alone is capable of stimulating the production of both 7S and 19S anti-γ-globulin antibodies in rabbits.

The highest titers and the most universal reactivity with individual rabbit antibodies were noted in the group of rabbits injected with autologous papain-split γ-globulin and in the ferritin-immunized animals boosted with autologous immune complexes or precipitates. However, some reactivity with rabbit antibody systems was also produced in rabbits immunized with autologous γ-globulin alone.

Of particular interest were the varying patterns of reactivity of specific antisera with

individual rabbit amboceptors and individual rabbit incomplete antibodies together with the inhibition of an individual rabbit incomplete antibody coat by certain rabbit γ-globulins and not by others. Moreover, individual rabbit γ-globulins did in some instances give weak precipitins with antisera obtained from papain-split or autologous γ-globulin-immunized animals. These marked differences in inhibition and in precipitation between individual rabbit γ-globulins suggest a specific role for the indivdual γ-globulins and possible participation of allotypic specificity in these reactions. However, attempts to relate such inhibitors to the known allotypes of both the incomplete rabbit antibody and its agglutinator have not been successful; pepsin digestion was found to destroy the inhibiting action of individual γ-globulins while preserving the allotypic specificity as reported by Kelus (16).

Papain-split rabbit γ-globulin also gave precipitins with various antisera and inhibitions of rabbit incomplete antibody coats. Whether this is at all related to individual γ-globulin specificities unmasked by splitting or whether it represents a group of antibodies directed towards new pieces of autologous digested γ-globulin, is not clear.

The fact that hyperimmunization with ferritin alone produced anti-γ-globulin antibodies of anti-rabbit specificity may mean that during immunization, combination of anti-ferritin antibody with antigen and its subsequent handling by the organism presents or unfolds sites on the rabbit γ-globulin molecule which can function as antigen. This is in line with certain of the concepts presented by Najjar(17). In somewhat analogous fashion, the buried potentially antigenic groups in the rabbit γ-globulin molecule either uncovered by prior enzymatic splitting or magnified by concurrent injection of Freund's adjuvants produce antibodies capable of reacting with rabbit γ-globulin. Considerable similarity in the patterns of reactivity of the antibodies produced by the different methods of immunization was observed in the present study, but whether the buried determinants functioning as antigens are identical or not remains to be proven. The results certainly indicate that tolerance to the animal's own γ-globulin is readily broken by the exposure to buried determinants under possible physiological conditions.

Enzymatically-split or slightly altered γ-globulin in immune complexes might also function as antigen in production of the human anti-γ-globulin factors, particularly those encountered in rheumatoid arthritis. Certainly the antibodies encountered in the rabbits showed many similarities to the human rheumatoid factors. In particular, the finding that individual variations in single rabbit γ-globulins, as noted in the inhibition of reactions with rabbit incomplete antibody, may be an important characteristic of such antibodies has a parallel in the iso- and autospecificity of human rheumatoid factors(18).

Summary. 1. Anti-rabbit γ-globulin antibodies were produced in the rabbit by simple immunization with horse spleen ferritin in Freund's adjuvant. Somewhat higher titers were obtained in these animals by further stimulation with autologous antibody-ferritin complexes. These antibodies were detected by the agglutination of rabbit cells sensitized with incomplete anti-G rabbit antibody and by sensitized sheep cell tests, using rabbit antibody as amboceptor. Antibodies reacting with human γ-globulin were also demonstrated by cells coated with incomplete Rh antibodies. 2. Low to moderate titers of anti-rabbit γ-globulin antibodies were also produced in rabbits immunized with autologous γ-globulin; considerably higher titers were noted when animals were immunized with autologous papain-split γ-globulin. Marked reactivity with human γ-globulin was also noted in these 2 groups of animals. 3. The anti-rabbit γ-globulin antibodies showed marked specificity for individual rabbit γ-globulins. This was demonstrated through reactivity with different red cell coats and in inhibition by some individual rabbit γ-globulins but not by others. Faint precipitins in agar gel also were observed between such antisera and some individual γ-globulins, as well as split products of rabbit γ-globulin. 4. These antibodies were both 7S and 19S in type and showed many similarities to human rheumatoid factors. Positive direct

and indirect Coombs' reactions were also encountered.

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Response of Germ-Free Animals to Experimental Virus Monocontamination. I. Observation on Coxsackie B Virus.* (28105)

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The demonstration of the pathogenicity of Coxsackie viruses for newborn suckling mice, by Dalldorf and Sickles(1), suggested that an animal with an immature defense system and poorly defined intestinal flora might be advantageous for the study of virus-host relationships. The germ-free animal provides the advantages of a relatively immature defensive mechanism, a sterile environment, and the absence of microbial flora in the intestinal tract.

Our purpose here is to summarize the results of experiments with germ-free, suckling mice challenged with Coxsackie B_1 virus, and the modification of the host's response to virus in relation to concomitant monocontamination with a species of bacteria. Comparative histopathologic changes in the animals are described elsewhere(2).

Materials and methods. Lobund (N.D. #1) Webster Swiss mice maintained in sterile isolators for 10 to 16 generations were available for the studies. Animals in plastic isolators, as designed by Trexler(3), were maintained on germicide-free San-i-cel bedding (Paxton Processing Company, Inc., Paxton, Ill.) and 50-10-C ration obtained from the Ralston Purina Co., St. Louis, Mo. Environmental temperature was stabilized at 25.5°C, and a light cycle of 12 hours "on" and 12 hours "off" was followed. The microbiologic procedures used in detection of bacteria, fungi, and protozoa were performed in accordance with the plan described by Wagner(4). Twenty-four-hour suckling animals were inoculated intraperitoneally with a 0.05-ml dose of a $10^{-8.8}$, $10^{-7.8}$, or $10^{-6.8}$ dilution of Coxsackie B_1 virus. Dilutions of virus correspond, respectively, to 0.1, 1, and 10 LD_{50}

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