coma cells. The precipitin tests indicated that the C_3H mice give somewhat better anti-hapten antibody production than the Swiss, although all responded.

C_3H mice injected with human myosin developed antibodies that reacted with skeletal muscle and embryonal rhabdomyosarcoma in the same way as did rabbit antimyosin serum as demonstrated by the indirect fluorescent antibody technic(5).

The use of mice for antibody production would be of particular value where the antigen may be scarce. The small amount of total antigen (2-3 mg) required per animal compared to that for rabbit is an obvious advantage.

The ascites tumor technic is not limited to mice but can probably be used in rats or other animals in which a suitable ascites transplantable tumor can be induced. The choice of tumor may be important since the ability of the animal to produce fluid varies with the tumor cells injected. For example, Ehrlich cells (from Swiss mice) give more fluid in Swiss rather than in C_3H. The Ehrlich ascites cells also produced more fluid in the C_3H mice than did the Gardner lymphosarcoma cells due to the fact that the C_3H mice succumbed more quickly from this tumor, limiting the number of ascites fluid taps to only 3 or 4, whereas with the Ehrlich cells in the C_3H mice as many as 8 taps could be made. The Gardner lymphosarcoma although capable of producing fluid in C_3H gave none in the Swiss mice in our experiments.

The one disadvantage of the procedure is that all the animals eventually die from the tumor, and although animals can be tapped as much as 3-8 times with considerable production of fluids, long term secondary immunization cannot be carried out once the tumor has been introduced intraperitoneally.

Summary. Ehrlich ascites and Gardner lymphosarcoma tumor cells were employed to induce ascites fluid formation in mice. Two strains of mice injected with antigens (X_p-human γ-globulin, myosin, ovalbumin) mixed in Freund's adjuvant had the capacity to respond with antibody production. The antibodies developed to X_p-human γ-globulin reacted with the hapten portion as well as normal human γ-globulin. The mouse anti-human myosin serum behaved similarly to the rabbit anti-myosin serum when studied by the immunohistochemical method.

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Studies of Antibodies in Ascitic Fluid of Individual Mice. (27685)

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Intraperitoneal injections of immunized mice with paraffin oil-adjuvant mixtures have been shown to produce ascitic fluid containing high titers of antibody(1-3). Ascites production in several strains of mice as a response to such stimulation have been compared(4). Antibody in mouse ascitic fluid has also been demonstrated by other investigators(5-7). These procedures for obtaining substantial amounts of ascitic fluid over ex-

tended periods now make it possible to follow antibody titers of individual mice.

Materials and methods. 1. *Ascites inducing and immunizing procedures.* a. Employing incomplete Freund's adjuvant-saline mixtures ascites was induced in 8-9-week-old female mice, each weighing approximately 20 g, of inbred strains C_3H/HeN, AKR/LwN, C_3H_fB/HeN and C57L/HeN by the method previously described(2). Forty days after the initial ascites inducing injection, mice from which appreciable amounts of ascitic fluid could be recovered were selected for immunization with ovalbumin and sheep erythrocytes. Prior to immunization ascitic fluid was withdrawn from each mouse and tested for antibodies to sheep RBC. None were found. Two different schedules of immunization were used. Each ascitic mouse of the 4 strains studied received 2 subcutaneous injections, each containing 1 mg ovalbumin (salt-free, twice crystallized) mixed with 0.1 ml complete Freund's adjuvant spaced one week apart. Simultaneously in strains AKR/LwN and C_3H_fB/HeN each mouse received 0.1 ml of 2.5% suspension of washed sheep RBC intravenously and a second similar injection 50 days after the first. Mouse strains C_3H/HeN and C57L/HeN received similar sheep RBC injections except that the first one was given 4 weeks following the initial ovalbumin injection and the second RBC injection 28 days after the first. Antibody titers of the ascitic fluid of individual mice were determined every 5 days for ovalbumin and every 2 days for sheep RBC following initial immunization. To permit regular tapping of mice for ascitic fluid for determinations of antibody titers, 0.1-0.2 ml of ascitic fluid were withdrawn from each mouse at a single time.

b. In a second experiment, ascites and immunization were initiated simultaneously to determine if the antibody titer could be affected by the ascitic state of the mouse at time of immunization. To accelerate ascites 4 intraperitoneal injections (each containing a mixture of 0.25-ml suspension of heat-killed 4×10^9 *S. aureus* cells and 0.25 ml incomplete Freund's adjuvant) were given to each of 28 AL/N mice at 3-4-day intervals. Simultaneously with the initial ascites-inducing treatment, each mouse was injected subcutaneously with 1 mg ovalbumin in 0.1 ml complete Freund's adjuvant given twice at weekly intervals. Tapping of mice was begun 3 weeks post initial immunization and maintained on a regular 10-day schedule. Titers of ovalbumin were determined for individual mice wherever ascitic fluid was obtained.

2. *Methods for titering antibody.* a. Sheep red cell hemolysin titers of ascitic fluid were estimated colorimetrically by the procedure described in(8). The titers are expressed as the dilutions giving 50% hemolysis. Titers of <10 were not considered meaningful.

b. The quantitative precipitin test described in(9) was employed to measure the antibody nitrogen in a single sample of ascitic fluid from each of 5 mouse strains immunized with ovalbumin complete Freund's adjuvant mixtures. Antibody nitrogens were

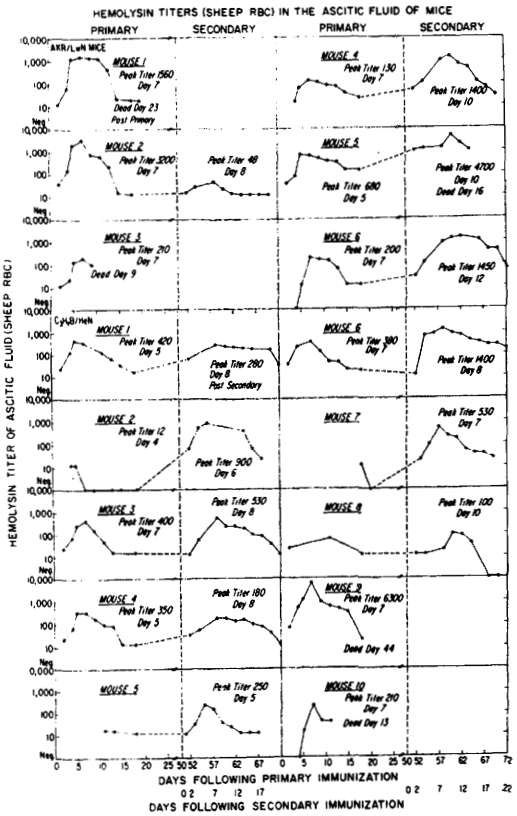

FIG. 1. Individual patterns of change in hemolysin titer of ascitic fluid of AKR/LwN and C_3H_fB/HeN mice following primary and secondary immunization with sheep erythrocytes.

also determined for successive samples of ascitic fluid taken from a single C_3H_fB/HeN mouse.

c. The mouse intralabial passive cutaneous anaphylaxis test (PCA) reported by Stone (10) was employed to titer ovalbumin antibodies in mouse ascitic fluid and consisted essentially of an intralabial injection of .05 ml of ascitic fluid followed one hour later by an intravenous injection of 0.2 ml of equal parts of 2 mg of ovalbumin and 0.4% Evans blue dye. A positive reaction was denoted by edema and blueing of the lip within 15 minutes following antigen injection. The titer of antibody is expressed as the highest dilution of ascitic fluid in 0.05 ml giving a positive reaction.

Results. Sheep erythrocyte hemolysin titers of ascitic fluid in 4 strains of mice (Fig. 1-2). The various panels of Figures 1-2 show individual antibody titers of ascitic fluid tapped from mice on prescribed schedules. These mice were already ascitic when the initial intravenous injection of sheep erythrocytes was given. Hemolysin titers were determined shortly after immunization and followed until the titer had disappeared. Reimmunizations with sheep erythrocytes were made after 28 days for C57L/HeN and C_3H/HeN mice (Fig. 2) and after 50 days for AKR/LwN and C_3H_fB/HeN mice (Fig. 1).

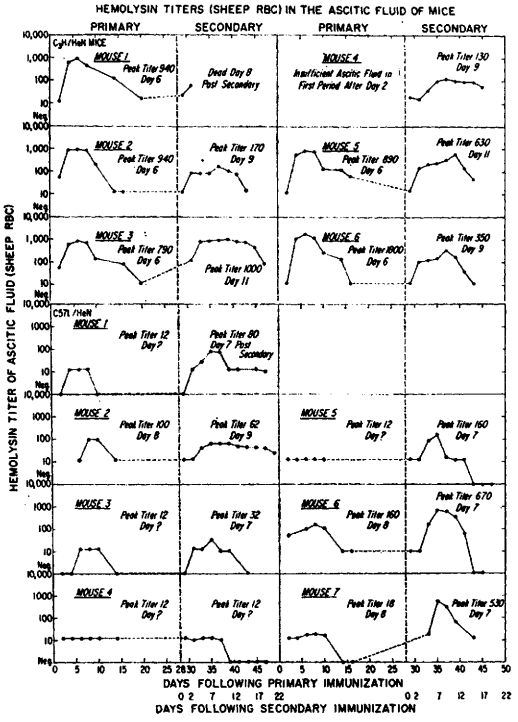

FIG. 2. Individual patterns of change in hemolysin in titer of ascitic fluid of C_3H/HeN and C57L/HeN mice following primary and secondary immunization with sheep erythrocytes.

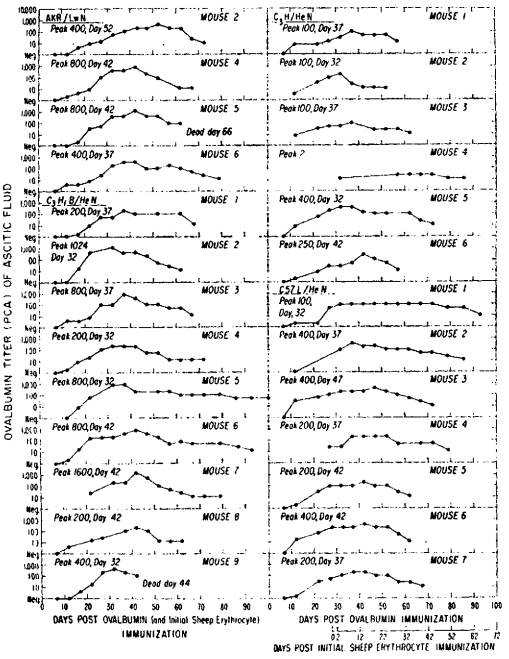

FIG. 3. Individual patterns of change in ovalbumin titer (PCA) of ascitic fluid for four strains of inbred mice. Titers shown refer generally to ascitic fluid obtained on a single day but may also refer to pools collected over a limited time period. Mouse numbers shown correspond to those given in Fig. 1-4 which show hemolysin titers (sheep RBC) for the same mice.

Employing generally a 2-day interval between determinations, antibody titers were evident 2 days after immunization. As seen in the figures wide variation is apparent between individual mice within a strain in the peak titers attained. Characteristically, however, rapid rises in titers are shown which attain their peaks within 7 days and are minimal after 15-20 days. Following preimmunization, peak titers tend to be higher, are attained later and diminish more gradually than after primary immunization. Some strain differences may also be manifest. For example, C57L/HeN initial peak titers are characteristically low and are more clearly evident following secondary immunization than follow-

ing primary immunization.

Ovalbumin titers of ascitic fluid determined by PCA reactions in 4 strains of mice (Fig. 3). For the same mice for which Fig. 1-2 show individual patterns of change of sheep RBC hemolysin titers, Fig. 3 shows the individual patterns for ovalbumin titers determined by PCA reactions. A few mice, where death had occurred early, are excluded. Individual mice are identified by number, permitting examination of the patterns of antibody titers obtained for sheep RBC and ovalbumin in the same mouse.

Individual patterns of ovalbumin titer characteristically show a somewhat gradual rise to a peak titer followed by an even more gradual decline. Characteristically, ovalbumin titers were positive 12 days after immunization, attained their peaks usually in 32-42 days post-immunization and persisted for extended periods. Although not shown in the figure, no positive titers were noted earlier than the 11th day. Individual ascitic fluids tested for ovalbumin antibodies by the double gel diffusion technic all showed single discrete lines.

Table I facilitates examination of the sheep RBC hemolysin and ovalbumin titers obtained for the different strains. The average peak titer (geometric mean) and the average day of peak following primary and secondary immunization are shown for each mouse strain. The range of individual peaks and days of peak are also shown. Average and individual peak ovalbumin titers were highest for the AKR/LwN and C_3H_fB/HeN strains where ovalbumin and sheep RBC immunization were made simultaneously. There was limited variation in time of attainment of peak titers, both within and between strains.

Ovalbumin titers of ascitic fluid of AL/N mice determined by PCA reactions. Fig. 4 shows the titers determined for the individual ascitic fluids from a group of 28 AL/N mice tapped at specific times. Subcutaneous immunization with ovalbumin-complete Freund's adjuvant mixtures was initiated simultaneously with the ascites-inducing treatment. Since the production of ascites did not become fairly regular until after about 5

TABLE I. Antibody Titers of Ascitic Fluid of 4 Inbred Mouse Strains.

Mouse strain	Total mice in exp.	Peak sheep RBC hemolysin titers: Primary immunization: No. of mice	Avg peak titer* (range)	Avg day of peak (range)	Peak sheep RBC hemolysin titers: Secondary immunization: No. of mice	Avg peak titer* (range)	Avg day of peak (range)	Peak ovalbumin titers (PCA): No. of mice	Avg peak titer* (range)	Avg day of peak (range)
AKR/LwN	7	6	510 (130-3200)	6.7 (5-7)	4	820 (48-4700)	10.0 (8-12)	4	570 (400- 800)	43.2 (37-52)
C_3H_fB/HeN	10	7	510 (12-6300)	6.0 (4-7)	8	380 (100-1400)	7.5 (5-10)	9	510 (200-1600)	36.4 (32-42)
C_3H/HeN	6	5	1020 (790-1800)	6.0 (6-6)	5	340 (130-1000)	9.8 (9-11)	5	180 (100- 400)	36.0 (32-42)
C57L/HeN	7	7	26 (12- 160)	8.0†(8-8)	7	100 (12- 670)	7.3†(7- 9)	7	240 (100- 400)	39.1 (32-47)

* Logarithmic averages (geometric means) shown for peak titers.
† Avg day of peak based on only 3 primary and 6 secondary peaks.

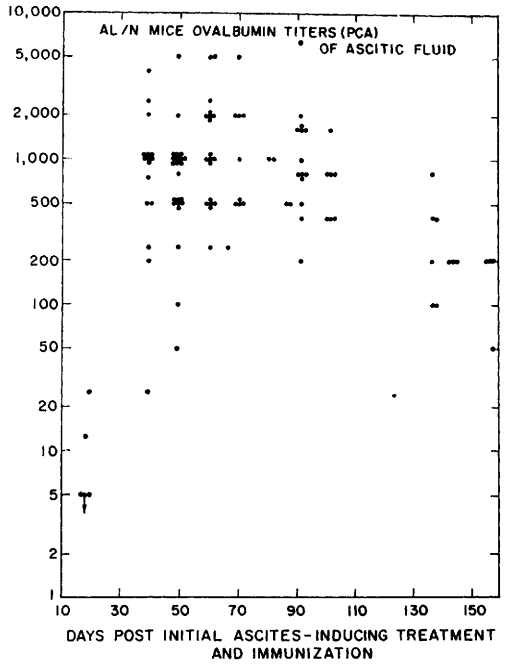

FIG. 4. Individual ovalbumin titers (PCA) of ascitic fluid for 28 AL/N mice. Arrow pointing downward indicates a positive titer of less than 5.

weeks, only a few early titer determinations were made. Fig. 4 shows all the individual ovalbumin titers determined by PCA titers obtained at specified times following immunization.

The limited number of titers determined 18 days post-immunization are low. The earliest subsequent titers obtained are 39 days post-immunization and indicate sharp increases in titer with some moderate rises after day 39. A fairly high range of titer remains over the interval of 39-101 days post-immunization. As of day 136 and later, titers are lower but have not disappeared.

Table II gives the ovalbumin antibody nitrogen determinations for 5 mice, one from each of the strains reported on in Fig. 3 and 4. Determinations were made for individual fluids obtained 42-47 days post-immunization and for the C_3H_fB/HeN mouse, determinations covered ascitic fluids obtained over a wide range in time. For the 42-47 day ascitic fluids the amounts of precipitating antibody nitrogen producing positive PCA reactions were low, 0.004-0.005 γ for the single mice of strains AKR/LwN and AL/N, and 10-fold greater, 0.04-0.06 γ, for the remaining strains. For the successively obtained samples from the C_3H_fB/HeN mouse, the range of variation was more extreme, attaining a value of 2.6 γ for the day 12 sam-

TABLE II. Antibody Nitrogen Content of Ascitic Fluids Yielding Positive Ovalbumin Titers (PCA).

	Ascitic fluid			
Mouse strain and No.*	Days post-ovalbumin immunization	Antibody nitrogen, γ/ml	Ovalbumin titer† (PCA)	Positive PCA antibody nitrogen* content, γ
AKR/LwN, #4	42	68	800	.004
C_3H_fB/HeN, #6	7	105	neg.	—
	12	105	2	2.6
	17	165	16	.5
	22	130	160	.04
	27	125	200	.03
	32	145	200	.04
	37	295	400	.04
	42	240	800	.015
	52	355	200	.09
	62	180	75	.12
	79	205	50	.20
C_3H/HeN, #5	47	110	100	.06
C57L/HeN, #3	42	255	200	.06
AL/N	42	102	1000	.005

* Mouse numbers shown correspond to those employed in figures.

† Ovalbumin titers shown are dilutions of ascitic fluid in 0.05 ml yielding a positive PCA reaction. Associated antibody nitrogen content is determined by dividing antibody nitrogen content of ascitic fluid, γ/ml, by 20 times the ovalbumin titer.

ple. The day 7 sample for this mouse yielded no positive PCA reaction with 7.5 γ precipitating antibody nitrogen.

Discussion. The results presented demonstrate the usefulness of employing mouse ascites as a tool for studying immunity in individual mice.

Working with mice already ascitic, well-defined individual patterns of rise and fall in hemolysin antibody titers for sheep erythrocytes following primary and secondary immunization were obtained. The data shown for mice C_3H/HeN #2 and #6 and AKR/LwN #2 are clear exceptions to the common experience that secondary peak titers are more pronounced than primary peaks. The significance of the low peak titers obtained for strain C57L/HeN remains to be investigated.

The delay in appearance of peak titers in the secondary response over that found in the primary response may possibly reflect the depletion of proteins in late ascites resulting from continuous production of ascitic fluid.

Studies on patterns of ovalbumin antibody titers in the current experiments necessitated employment of adjuvant to obtain measurable amounts of antibody. The suggested late attainment of peak titers and dimunition of ovalbumin antibodies is perhaps typical when antigens are administered with adjuvant.

To detect early changes in antibody titer, the experiments conducted suggest the need for working with mice already ascitic. This then raises the question of whether the patterns of change in titer are similar for both ascitic and nonascitic mice. In the ovalbumin titer studies made, the day of peak titer was approximately the same for the AL/N mice (immunized when nonascitic) as for the remaining 4 strains (immunized when ascitic). The greatest ovalbumin titers attained seem to be confined to the AL/N mice. The morbid condition of the ascitic mice at time of immunization may have interfered with the ability to form antibody.

An extremely wide range in variation in amount of antibody nitrogen associated with a positive ovalbumin titer determined by PCA was noted. The smallest amounts of antibody nitrogen associated with a positive reaction, 0.004-0.005 γ agree substantially with the findings of Munoz and Anacker(5) and indicate high sensitivity for the mouse PCA reaction. But the substantially higher nitrogen levels found in some instances, and the finding of a negative PCA reaction at high antibody nitrogen levels in early antibody production in ascitic fluid, would indicate that there is no direct proportionality between the amount of precipitating antibody in terms of time and the limiting dilution for a positive PCA reaction. It has been shown in the rabbit that the first antibodies to appear following immunization with a single injection of protein were of the 19S variety with the 7S variety appearing 3-4 days later (11). Others have shown that the skin binding antibodies necessary to give a positive PCA were of the 7S variety(12). Our findings of negative PCA's in the presence of large amounts of precipitating antibody nitrogen in early antibody production indicate the necessity of characterizing the antibodies in the mouse in terms of time following immunization.

Summary. Employing mice with ascites, individual patterns of change in hemolysin and ovalbumin titer of ascitic fluid were studied for 4 inbred strains of mice. Mice received both primary and secondary immunizations of sheep erythrocytes. There was evidence of antibody titers 2 days after immunization. Characteristically, after primary immunization, rapid rises in titer occurred which attained their peaks within 7 days and were minimal after 15-20 days. Following secondary immunization, peak titers usually were higher, attained later and tended to diminish more gradually than following primary immunization. Some strain differences in the pattern of antibody titer changes were manifest. Ovalbumin titers measured by the PCA reactions were evident from the 12th day, increased gradually to a peak, generally 32-42 days after immunization, then diminished more gradually thereafter.

In work with AL/N mice, not ascitic at time of immunization, the early rise in titer was approximately the same as for the other strains that were ascitic and individual titers persisted for as long as 110 days post-im-

munization. Individual titers were higher for AL/N mice than for the other strains, perhaps due to their non-ascitic state at time of immunization. Determination of precipitating antibody nitrogen in the ascitic fluid of ovalbumin-immunized mice indicated that early levels of antibody nitrogen as high as 105 γ/ml did not produce a positive PCA reaction sooner than 11 days post-immunization. The need for further studies on the characterization of antibodies in time of their appearance is indicated.

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Serologic Typing of *Listeria monocytogenes* by Gel Diffusion Using Thermostable Antigens. (27686)

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Listeria monocytogenes presents a complex antigenic structure composed of both flagellar and somatic antigens. Early investigators (1-7) classified the organisms serologically into 2 types based on animal origin (*i.e.,* rodent or ruminant). Paterson(8,9) demonstrated the presence of somatic O-antigens and flagellar H-antigens, and on the basis of their somatic and flagellar response distinguished 4 serologic types (*i.e.,* I, II, III, and IV), the type having no relation to its origin. Seeliger(10) confirmed the work of Paterson, further distinguishing 2 subtypes in type IV strains, designating them IVa and IVb.

The use of the typing scheme based on O- and H-antigenic factors requires specially absorbed sera. Too often the results can be erroneous if not accomplished by trained workers. Seeliger(10) has pointed out that precipitin and gel diffusion technics, although requiring high-titer serum, present fewer cross reactions than are observed in agglutination tests if extracts of the organisms are used. Recently Smith and Metzger(11) demonstrated capsule production in *L. monocytogenes.* This investigation was made to determine the antigenic components of *L. monocytogenes* by gel diffusion, taking into consideration the thermostable O-antigens as well as those possibly produced by an enhanced capsule.

Materials and methods. Immune sera. Strains ATCC 4428(I) and Seeliger's strains 5348(II), 5105(III), 5214(IVa), and 1071/53(IVb) were subcultured twice on trypticase soy agar containing 10% rabbit serum and 5% glucose to enhance capsule production (SGTSA)(11). Ten flasks, containing 100 ml of trypticase soy broth plus 10% serum and 5% glucose (SGTSB) were then inoculated from the SGTSA and incubated for 18 hours at 37°C in a rotator incubator. The cultures were centrifuged in the cold at 2,000 rpm, and the supernate discarded. The sediments of each type were pooled and sus-