not think such an explanation is completely satisfactory. An alternative explanation is offered by Berglund and Fagraeus to explain the restoration by intact spleen cells of antibody producing capacity in rats previously treated with cortisone(10). These authors suggest that added cells or cell fractions may contribute to a preliminary processing of antigen to enhance its employment by immunologically competent cells.

Summary. Normal guinea pig spleen cells, when injected with bovine plasma albumin or egg albumin, appear to enhance the delayed sensitivity to the antigen which appeared early in the course of the immunologic response in guinea pigs.

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Ascites Tumor Fluid as a Source of Antibodies in Mice. (27684)

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Recent studies by Munoz(1) have shown that antigens mixed with Freund's adjuvant and injected intraperitoneally into mice caused the development of large amounts of peritoneal fluid containing specific antibody in high concentrations. Difficulty is observed in that ascites formation does not always occur particularly with mice of certain strains (2) and time of production of ascites and amount formed is variable(1).

Herrmann and Engel(3) showed that ascites fluid induced by Sarcoma 180 cells in mice immunized with a viral preparation contained antiviral antibodies. Lieberman *et al.* (4) has employed *Staphylococcus aureus* strain 18-adjuvant mixtures in mice for ascites formation.

Since many transplantable ascites tumors are available in mice and since mice implanted with ascites tumors invariably develop ascites with large amounts of fluid, mice should prove very useful in producing antisera to small quantities of antigen. Use of ascites tumor has the advantage over the use of adjuvant alone in that ascites can be produced with greater certainty, and by regulation of cell dosage the time of ascites formation can be regulated. The results of a study of the production of precipitating antibodies by this procedure are reported here.

Methods. Mice of the Swiss/Webster and the C_3H/Heston strain and of both sexes were injected intraperitoneally with 0.25 ml of an adjuvant antigen mixture, reinjected with a second 0.25-ml dose 2 weeks later, then at the appropriate time injected with approximately 35 million Ehrlich ascites cells or Gardner lymphosarcoma ascites cells. Animals were tapped starting one week after ascites cells were introduced.

The adjuvant contained 50 ml Bayol F, 100 mg mycobacterium butyricum and 25 ml Arlacel 80 and was sterilized in an autoclave before use. The antigen preparation contained 2 volumes of antigen solution mixed with 3 volumes of adjuvant.* The antigens

* The procedure is similar to that described by Munoz(1) with slight changes in materials.

used were chicken ovalbumin; human myosin (5) and human γ-globulin coupled with diazotized p-aminobenzoic acid (hereinafter called X_p-human γ-globulin).

The coupling conditions were described by Nisonoff *et al.*(6). Ovalbumin coupled with diazotized p-aminobenzoic acid (X_p-ovalbumin) was prepared similarly by Mr. G. Radzimski.

For ascites formation either the Gardner lymphosarcoma in the ascites form or the Ehrlich ascites tumor were employed. Cell dosage was regulated so that maximally tolerated ascites formation would occur at about 7 days after the last injection. The animals were tapped with a No. 18 needle under light ether anesthesia and approximately 4-5 ml was obtained at each tapping (as much as 25 ml obtained in a single tapping). Tappings were made from day to day or at 2-3-day intervals. Sufficiently large pools of antiserum were obtained in this manner. All animals eventually died from the tumor growth 2 to 3 weeks after implantation of the tumor.

The amount of specific precipitable antibody present in the anti-ovalbumin and anti-X_p sera was assayed by determining the maximum amount of precipitate obtained with ovalbumin or X_p-ovalbumin respectively. The precipitated protein was determined by Kjeldahl analysis.

The indirect fluorescent antibody procedure was employed to study the mouse anti-human myosin serum. Rabbit anti-mouse globulin was labeled with fluorescein isocyanate. Sections of embryonal rhabdomyosarcoma and normal human skeletal muscle were stained and compared with the results reported for rabbit anti-human myosin serum (5).

Results. Antiserum prepared against ovalbumin in Swiss mice contained 1.9 mg antibody/ml fluid precipitable by antigen. 168 ml of cell free ascites fluid were recovered after approximately 7 tappings per mouse for the 6 mice used in this group. It is of interest (Table I) that the Ehrlich ascites tumor induces slightly more fluid in the Swiss than in the C_3H mice. It appears that larger volumes of fluid can be obtained if older mice are used and maximum turgidity is allowed to occur in the animal before tappings are made rather than by making frequent tapping at shorter intervals. Both strains responded with antibody production as determined by precipitin tests (Table I).

Antiserum prepared in C_3H mice injected with X_p-human-globulin gave 200 γ of anti-hapten antibody/ml of fluid as precipitated with the X_p-ovalbumin antigen. (The antiserum was also capable of reacting with normal human γ-globulin.) Here again the Ehrlich ascites cells produce more fluid in the C_3H mice than do the Gardner lymphosar-

TABLE I. Composite Summary of the Mouse Strains Used, Amount of Antigen Given, Volume of Fluid Produced, and Qualitative Precipitin Results.

Antigen employed	Mouse strain and number/ group	Ascites cells used for fluid induction*	Total amt of antigen given per animal	Vol of fluid/ animal†	Precipitin reaction
			mg	ml	
X_p-Human γ-globulin	C_3H/10	Ehrlich	2.72	30	+ (200 γ antibody protein/ml)
" "	C_3H/8	Lymphosarcoma	3.0	6	w+
" "	C_3H/5	Ehrlich	7.0	13.9	w+
" "	Swiss/8	Ehrlich	3	24.75	w+
" "	Swiss/9	Ehrlich	3	47.7	w+
Ovalbumin	Swiss/6	Ehrlich	2	28	+ (1.9 mg antibody protein/ml)
"	C_3H/6	Lymphosarcoma	3	8.5	+
Human myosin	C_3H/5	Ehrlich	1.74	25	+

* In every case 100% ascites formation occurred.
† Total volume of fluid obtained/No. of mice in group.
w+ Weak precipitation in ring test, or agar diffusion tests.

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-