extensive necrosis and liquefaction of the brain. This mouse brain adapted strain of RS virus was found to be strictly neuropathic, in that it produced lesions only in the nervous system of the intracerebrally inoculated suckling mice and failed to produce either infection or lesions in any of the extracerebral tissues which were examined.

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An Enhancing Factor in Normal Chicken Serum Augmenting Saline Agglutination of a Mycoplasma Antigen-Antibody System.* (31924)

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During experiments with agglutination tests involving antisera against *Mycoplasma gallisepticum* (MG), it was noted that the addition of serum from apparently normal chickens augmented the saline agglutination by 2-3 doubling dilutions, whereas serum from turkeys had no effect. Saline and antiglobulin determinations on these normal chicken sera failed to demonstrate antibodies directed against MG.

A review of the literature failed to reveal previous studies of a factor in normal chicken serum that would enhance agglutination. However, Makinodan *et al*(5) had demonstrated a macroglobulin in normal chicken serum that coprecipitated with antibody against bovine serum albumin (BSA). This coprecipitation occurred with aged normal serum or in the presence of 1.5 M NaCl, whether the serum was aged or not. Similarly, Orlans *et al* (6) showed that addition of normal fowl serum increased the amount of specific precipitate with anti-BSA serum. The yields of precipitate were greater with 0.9% NaCl than with 8% NaCl. Subsequently, Orlans and Rose(7) determined that the increased precipitation attributed to normal serum was due to one or more factors, including a macroglobulin. These factors were heat-stable (56° for 30 minutes) but were destroyed at pH 5.5 or lower and were inhibited with ethylenediaminetetraacetic acid (EDTA). The present communication deals with some of the characteristics of agglutinating enhancing factor (EF).

Experimental. Antigens. The antigens employed were as follows: 1) *M. gallisepticum* prepared by a procedure described previously (1); 2) similarly prepared antigens from a nonpathogenic avian mycoplasma strain designated simply as Tu; 3) *Salmonella typhimurium* "O" antigen prepared by a standard method(4); 4) *Haemophilus gallinarum* antigen (obtained from Dr. Richard Yamamoto of this Department); and 5) washed avian red blood cells collected from Single-Comb White Leghorn chickens (SCWL).

Antisera. MG antisera were prepared in chickens and turkeys; the other antisera used were of chicken origin. Antibodies directed against MG were prepared by nasal or tracheal infection of chickens and turkeys with live organisms, whereas Tu antisera were raised by the intravenous inoculation of live

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organisms. Washed chicken erythrocytes and Salmonella "O" antigen were similarly inoculated by the intravenous route for specific antibody production. Antisera against *H. gallinarum* were prepared by inoculation of live organisms into the infraorbital sinus of chickens, followed later by intravenous inoculations.

Experimental birds. The chickens used were from the Poultry Husbandry Department, University of California, Berkeley. They were obtained as day-old chicks and reared in isolation. Surveillance and examination by poultry pathologists of the University have shown that these fowls are free of known viral or bacterial diseases. No vaccination of any kind has been practiced in the parent flock. The turkeys used were commercial Broad Breasted Bronze or Large Whites known to be free of MG for many generations.

Normal sera. Normal sera taken by venipuncture from chickens, turkeys, and rabbits of various ages were inactivated for 30 minutes at 56°C and absorbed overnight with the test antigen (*i.e.,* serum to be tested for EF activity on an MG agglutination system was absorbed with MG antigen). Saline and antiglobulin agglutination tests were then conducted on each absorbed serum to ensure that no demonstrable antibody activity existed for the test system in question. A few of the normal sera with EF activity were also treated with EDTA (2 mg/ml).

Antiglobulin serum. Mature white rabbits were given 1 ml of whole normal chicken serum mixed with an equivalent amount of Freund's complete adjuvant at multiple intramuscular sites. Three weeks later, an additional quantity of serum (without adjuvant) was injected intraperitoneally; this was repeated 2-3 times more at intervals of 7 to 10 days. The animals were exsanguinated 3 to 4 weeks later, and the pooled serum was tested for antiglobulin activity by microimmunoelectrophoresis and by the antiglobulin test using MG.

Demonstration of EF activity. Doubling dilutions of pooled chicken MG antiserum were mixed with MG antigen by procedures described previously(2,3) to determine antiglobulin (Coombs-type reaction) agglutination end-points. After 2 hours of incubation at 37°C, the antiserum dilutions plus antigen were held overnight at 5°C; each tube was centrifuged (1,200 × *g*), and the sediment (MG antigen with attached globulins) was washed 3 times in physiological saline to eliminate free, unattached globulins. Each tube of reactants was then resuspended to a volume or multiples thereof as previously determined(2,3).

One drop from a Pasteur pipette (0.025 ml) of each dilution was placed on a glass plate and rotated gently for 3 minutes. Agglutination was recorded in degrees ranging from 4+ (complete agglutination) to 1+ (a barely visible reaction). The highest antiserum dilution representing a reaction intensity of 2+ or greater was taken as the agglutination end-point. To assure that the agglutination system had an agglutination end-point which could be augmented by an additional factor, the agglutination system was again placed on a glass plate as above, and to each drop (one from each dilution) was added an equivalent drop of rabbit antichicken serum. This antiglobulin agglutination titer was determined as described above.

The procedure was repeated again, as for the antiglobulin test, but adding instead one drop of EF sera to each drop of antigen-antibody dilution. Agglutination was again assessed, and the end-point was taken as the enhanced titer.

Normal turkey and rabbit sera were similarly tested for EF activity.

Enhancing activity on other agglutinating systems. Having established that certain sera contained the capacity to enhance an MG antigen-antibody system, their ability against *M. meleagridis,* Tu mycoplasma, *S. typhimurium,* and *H. gallinarum* was determined in a similar manner. Activity on an RBC system was conducted as follows: 0.025 ml of 3-times-washed chicken RBC's of a 0.5% suspension in 0.85% NaCl was added to an equivalent quantity of doubling dilutions of a chicken anti-RBC serum. The degree of agglutination was recorded after 3 minutes of rotation on a glass plate. This value was taken as the saline agglutination end-point. To assess the enhanced and antiglobulin agglutination titer,

TABLE I. Enhancing Effect of Normal Sera on *M. gallisepticum* Agglutination.

EF serum #	Turkey antiserum AS-7T*			Chicken antiserum AS-14C*			Chicken antiserum AS-17C*		
	Saline	Enhanced	Anti-globulin	Saline	Enhanced	Anti-globulin	Saline	Enhanced	Anti-globulin
1	160†	640	5120	80	80	1280	160	320	5120
2	160	640	5120	80	80	1280	160	320	5120
3	160	320	5120	80	80	1280	160	320	5120
4	160	320	5120	80	80	1280	160	320	5120
5	160	1280	5120	80	320	1280	160	1280	5120
6	160	320	5120	80	80	1280	160	320	5120
288	—‡	—	—	80	320	640	—	—	—
291	—	—	—	80	320	640	—	—	—
1523	—	—	—	40	320	640	160	1280	5120
497	—	—	—	40	160	1280	160	640	5120

* Pooled MG positive antiserum. † Reciprocal of the serum dilution. ‡ Not done.

equal quantities, usually 0.5 ml, of RBC suspension and antiserum dilutions were mixed and allowed to react overnight at 5°C. Each tube was washed 3 times and resuspended to its original volume in physiological saline. A drop from each dilution was placed on a glass plate, and an equal amount of rabbit anti-chicken serum was added. After 3 minutes of rotation, this antiglobulin agglutination value was recorded. The procedure was repeated, but with the EF sera instead.

Results. Pooled MG chicken and turkey antisera were used to determine the presence of EF in 10 chicken sera. Agglutination was enhanced in every case with a turkey antiserum pool (AS-7T) and with one chicken antiserum pool (AS-17C). However, a pool of chicken antiserum (AS-14C) failed to have agglutination enhancement with 5 of the EF sera tested (Table I). Sera 288 and 291, while enhancing the agglutination reaction of pooled chicken serum AS-14C, did not increase agglutination with *H. gallinarum, S. typhimurium,* or chicken RBC's. The Tu mycoplasma agglutination system was increased 8-fold (Table II).

Heat or EDTA treatment of the normal serum did not reduce EF activity more than one dilution.

Sera from 10 normal turkeys and 5 rabbits had no EF activity when tested against MG with pools of chicken or turkey antisera.

Discussion and summary. Enhancement of agglutination with antibody of chickens against MG with a heat-stable component of normal fowl serum is in accord with the studies of Orlans and Rose(7). In the research of Orlans and Rose(7) and Makinodan *et al*(5) bovine serum albumin was the antigen, and precipitation increased with normal fowl serum. In the present paper, limited studies with two bacterial antibodies and antired blood cell serum failed to exhibit increased agglutination in the presence of EF in normal serum. These same normal sera enhanced the agglutination reaction with two unrelated Mycoplasma, *M. gallisepticum* and Tu. This observation would possibly suggest some

TABLE II. Enhancing Effect of 2 Normal Chicken Sera on Agglutination of *H. gallinarum, S. typhimurium,* Tu Mycoplasma, and Chicken Erythrocytes.

EF serum #	Agglutination method	Antigen-antibody system			
		H. gallinarum	*S. typhimurium*	Tu mycoplasma	RBC
288	Saline	40*	320	20	80
	Enhanced	40	320	160	80
	Antiglobulin	640	640	320	640
291	Saline	40	320	20	80
	Enhanced	40	320	160	80
	Antiglobulin	640	640	320	640

* Reciprocal of serum dilution.

relation between Mycoplasma antibody and the normal enhancing factor in serum.

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Thyroid Effect on Birthweight in C 57 BL Mice and *Peromyscus*.* (31925)

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Conflicting evidence exists concerning the influence of thyroid hormones on birthweight in mammals. Thyroid hormone administered to pregnant females is reported to increase birthweight in rabbits(1), but decrease it in guinea pigs(2) and possibly rats(3). There are reports that goitrogens administered during pregnancy decreased fetal weight in rats(3,4) and birthweight in the guinea pigs (5). Maternal thyroidectomy reportedly reduced birthweight in C 57 mice(6), but other findings suggest that neither thiourea treatment nor thyroidectomy of pregnant rats materially effected neonatal weight(7,8).

To date there have been no attempts to critically evaluate the influence of physiological levels of maternal thyroid hormone on birthweight, particularly with reference to a known thyroid secretion rate (TSR) estimate. This prompted us to examine the effect of thyroid hormone upon weight of newborn in 3 forms of rodents where TSR data were available: C 57 BL laboratory mice *(Mus musculus);* deermice *(Peromyscus maniculatus bairdii);* and oldfield mice *(P. polionotus).* If the thyroid contributes significantly to birthweight in these rodents, experimental alterations of hormone levels within a subtoxic range should produce a measurable effect.

Materials and methods. The experimental procedures with C 57 BL mice were somewhat different from those with *Peromyscus.* This was necessitated by inherent differences in the stocks, particularly with regard to genetic variability. Further, it was thought that different approaches to the same problem might render the findings more conclusive.

C 57 BL mice of the 6 J line were employed because of the vigor, low tumor incidence and genetic homogeneity of the strain. Average adult TSR's estimated for this strain by the I^{131} depletion method(9) and by goiter prevention technique(10) vary from 3.33 to 4.80 μg l-thyroxine/100 g bw/day.

Nineteen mated C 57 BL pairs were established using 90-120-day-old animals. Pairs were divided into 4 groups, maintained under controlled conditions of light and temperature, fed and watered *ad libitum.* Pregnant mice from Group 1 received daily injections of l-thyroxine solution s.q. at the estimated normal female TSR (3.33 μg/100 g bw), and those in Group 2 received twice the estimated TSR. In both groups endogenous thyroid function was blocked utilizing .01% propylthiouracil (PTU) solution as drinking water. Pregnant mice in Group 3 were given PTU only, and those in Group 4 received a sham injection of distilled water daily. Only the second and third litters from each mated pair were used to obtain birthweight data.

Two varieties of *Peromyscus* were laboratory bred from mice 10 or more generations

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