that $k_3 > k_4$, so that labile fluoride is rapidly excreted. Nothing is known at present of the relative magnitudes of k_4 and k_5 or of the toxic consequences of a rapid turnover of fluoride through tissues in which only a trace amount may be found at any one time. Conceivably these equilibria could be altered by pathological conditions, particularly by renal disorders, which would decrease the constant k_3 in relation to k_4, or by endocrine dysfunction, causing increased turnover of skeletal constituents, an increase in k_2, with corresponding rise in level of circulating plasma fluoride. Investigation of these dynamic aspects of fluoride metabolism clearly is needed.

Summary. Administration of 1 mg F daily for 90 days to weanling rats failed to demonstrate any increase in fluoride content or concentration of heart, liver, or kidney, even as fluoride saturation of the skeleton was approached. Fluoride content of the carcass, femur, and sternum increased markedly with fluoride ingestion but rate of retention decreased throughout the experiment, particularly after attainment of skeletal maturity. Although no increase in soft tissue fluoride was demonstrated, the toxic effect of this level of fluoride (1 mg per rat per day) was demonstrated by the decreased weight gain of the fluoride animals when compared to the control group.

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Serum Gamma Globulin and *Trypanosoma cruzi* Agglutinin in Embryonic, "Normal" and Germ-Free Chickens.* (24262)

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The transmission of maternal serum proteins and antibodies to chick embryos *via* the yolk has been established by various investigators(1-3). The serum proteins of the developing embryos show progressive changes with age; Marschall and Deutsch(2) found low levels of gamma globulin in the serum of the 11-day embryo; later this serum fraction appeared in increasing amounts until after hatching. Brandly *et al.*(1) reported the absence of Newcastle virus antibody in the sera of 11-day embryos; they showed significant amounts of this antibody in the sera of 15-day-old, and high titers in the sera of 18-day-old embryos. Similar developmental patterns of *Trypanosoma cruzi* agglutinin in the sera of developing chick embryos was demonstrated by Borsos and Warren(4). It was also shown that germ-free chicks 86 days of age or older were devoid of detectable *T. cruzi* antibody. In contrast, all "normal" adult controls contained this factor in high titer. Recently, it was reported that the sera of 8 to 17-week-old germ-free chickens contained less gamma globulin than the "normal" controls; however, no difference was found between "normal" and germfree birds 4 to 8 weeks of age (5).

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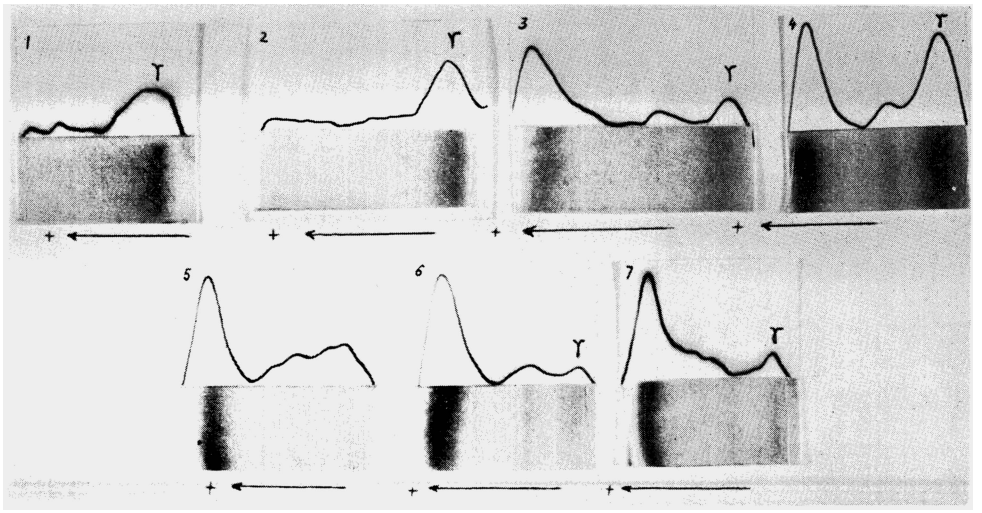

FIG. 1. Electrophoretic patterns of chicken sera. 1—15-day-old embryo. 2—2-day-old "normal" chick. 3—28-day-old "normal" chick. 4—"normal" adult. 5—24-day-old germ-free chick. 6—30-day-old germ-free chick. 7—313-day-old germ-free chick.

Considering these results, a correlation was expected between *T. cruzi* agglutinin and serum gamma globulin levels in chickens.

The purpose of this work was to study by paper electrophoresis the gamma globulin content of sera from developing embryos and from "normal" and germ-free chickens of different ages. Simultaneously, the presence of *T. cruzi* agglutinin in these sera was determined and correlated to gamma globulin content.

Materials and methods. The brasil strain of *T. cruzi* was maintained in culture as described by Warren(6). The technic for antibody assay will be described elsewhere in detail. Briefly, 1/10 dilutions of sera inactivated at 56°C for ½ hr. were tested against the culture forms of the organisms; the mixtures were sampled at time intervals and observed in the light microscope. 4+ denotes agglutination of all organisms, 0 denotes no visible agglutination at 90 minutes.

"Normal" sera were obtained from blood of laboratory hatched and reared White Leghorn chickens. The animals were bled by heart puncture or from the wing vein. The tubes with the clotted blood were stored usually overnight at 4°C; after centrifugation the sera were stored at –20°C.

Embryonic blood was obtained from a blood vessel of the chorioallantoic membrane and was handled similarly to adult chick sera(3).

Paper electrophoresis was performed in a cold room at 4°C with a Na-barbital-barbituric acid buffer at pH 8.6, ionic strength 0.1 for 5 hrs. under 400 volts and 10 milliamps (7).

After electrophoresis the papers were stained with brom-phenol blue. The protein patterns were photographed from the paper strips with an automatic recording photometer‡ and integrated. Since all the electrophoretic analyses were performed under the same conditions and the strips treated the same way, it is possible to compare the variations of gamma globulin levels to the adult "normal" sera.

Sera of germ-free chicks were generously supplied by Dr. T. G. Ward, Lobund Inst., Univ. of Notre Dame. They were shipped in dry ice and were stored at –20°C.

Results. A total of 46 sera were analyzed for gamma globulin content and *T. cruzi* agglutinating activity.

Fig. 1 presents a few typical electrophoretic patterns of sera from animals of different ages and rearing conditions. It is seen that the serum fractions vary extensively with the age of the donor bird. For this reason, in

‡ From Laboratories LERES, Paris, France.

TABLE I. Gamma Globulin Content and *T. cruzi* Agglutinin Indices of Chicken Sera.

Embryonic															
Age, days	11	11	15	15	15	18	18	18							
% gamma g.	5	25	15	35	20	15	20	15							
Aggl. index	0	0	1+	1+	1+	2+	1+	2+							
Young "normal"															
Age, days	2	2	2	5	5	5	22	22	22	22	22	28	28	28	28
% gamma g.	50	33	85	20	70	60	60	60	30	35	20	20	35	20	15
Aggl. index	3+ to 4+			n.d.	3+	4+	2+	2+	2+	1+	2+	1+	0	0	1+
Young and adult germ-free															
Age, days	24	30	30	35	46	49	49	49	86	283	283	283	313	313	313
% gamma g.	30	20	10	30	15	30	30	110	35	10	15	5	15	15	30
Aggl. index	1+	1+	1+	1+	0	1+	0	3+	0	0	0	0	0	0	0
Adult "normal"															
Age, days	180 to 500														
Area gamma g.	19	23	20	19	18	23	14	23	Avg area: 20*						
% gamma g.	95	115	100	95	90	115	70	115							
Aggl. index	4+	4+	4+	4+	4+	4+	4+	4+							

* Avg area of 20 was taken as 100% gamma globulin content.

comparing gamma globulin contents of the different sera, the amount of gamma globulin in each serum was expressed in terms of percentage of the average gamma globulin content of the 8 "normal" adult controls which was arbitrarily taken as 100%.

Table I records gamma globulin content of each serum together with the results of the *T. cruzi* agglutination tests. The data show that high gamma globulin contents were associated with high *T. cruzi* agglutinin indices. Sera of embryos were low in gamma globulin content; they also showed low agglutinating activity. Newly hatched birds contained gamma globulin levels up to the range of the "normal" adults; their agglutinin indices were correspondingly high.

The gamma globulin of the embryos and the newly hatched birds stemmed from the mother hen. By the 28th day after hatching gamma globulin levels (and agglutinating activity) dropped to low levels; in fact, some birds lost their agglutinating activity. After immunological maturity was reached all tested "normal" birds showed high gamma globulin contents with 4+ *T. cruzi* agglutinin indices. The gamma globulin in sera from germ-free birds continued to decline and stayed at low levels even in the adults. We have found one bird (age 49 days) which had a high gamma globulin level in its serum and also elevated *T. cruzi* agglutinin index. It is possible that this bird retained its maternal gamma globulin for an unusually long time after hatching; the alternative possibility of contamination must also be considered, although the bird tested free of contaminants at the time the serum was obtained (Personal communication, T. G. Ward.)

Conclusions and summary. The progressive changes in gamma globulin content in the sera of developing embryos indicated here confirm earlier findings(2). Following absorption of the yolk by the newly hatched chick, the transmitted gamma globulin levels decline until the young birds attain immunological maturity. The mature birds show high gamma globulin levels as illustrated by the electrophoretic patterns. In contrast, germ-free chicks reveal low levels of gamma globulin which remain so even at 313 days of age; *T. cruzi* agglutinin follows the same pattern. These results strongly suggest that a close correlation exists between serum gamma globulin levels and the indicator antibody. They also suggest that chickens reared under germ-free conditions, after losing the maternal antibody, do not replace it with gamma globulin of their own to any significant degree. Further studies are needed in order to follow the serum protein changes in older adult germ-free chickens and their response to controlled antigenic stimuli.

We are indebted to Drs. L. G. Warren and Rodrigo Zeledon for supplying the *T. cruzi* cultures used in this study.

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Susceptibility of Cortisone-treated Mice to Infection with Mouse Hepatitis Virus.* (24263)

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The strain of mouse hepatitis virus (MHV) used has been described by Gledhill(1-3). It is the virus from which the enhancing agent, *Eperythrozoon coccoides,* has been excluded. This virus causes a mild, rarely fatal, infection in weanling Swiss mice and a fatal infection in suckling mice. In the latter case, focal necrotic lesions appear in the liver and evolve into diffuse, cirrhosis-like lesions. It was found that normally resistant weanlings became susceptible to fatal hepatitis infection when subjected to cortisone treatment. In this respect, cortisone has been used extensively to change the native resistance of various animals and embryonated eggs to infection by protozoan, mycotic, bacterial, rickettsial, and viral agents(4).

Materials and methods. Weanling Swiss mice, 8-10 g, and 1-2 day-old suckling mice, were used routinely. Stock virus was made from infected suckling-mouse livers as a 10% emulsion (w/v) in medium 199 or Hank's solution containing 0.2 mg/ml streptomycin and 200 units/ml penicillin. Liver preparations were clarified by centrifugation at 8,000 rpm for 20 min at 4°C, sealed in ampoules, and stored in a dry ice chest. The usual inoculum (0.05 ml) administered *via* the peritoneal route to weanling mice contained $10^{-3.5}$ suckling mice ID_{50}s(5) of MHV. Cortisone acetate (Upjohn Co., aqueous suspension, 25 mg/ml) was injected intramuscularly in single doses of 1.25 mg unless otherwise indicated. Mice were observed daily for 6 to 15 days. Survivors were killed with ether and autopsied. Livers showing lesions of questionable significance were homogenized in saline (10% w/v) and assayed in suckling mice.

Results. Large doses of cortisone alone caused some deaths, ascites, and distinct, pinpoint, whitish liver lesions in a small proportion of mice (Table I). In a series of 2 experiments, a total of 25 mice were given a single injection of cortisone (1.25 mg) and a total of 31 mice were given 4 daily injections of cortisone (total 5.0 mg). Observations were made daily for 12 and 15 days (Table I). Survivors were sacrificed and examined for evidence of liver damage and ascites. Of the mice which received 1.25 mg cortisone, an average of 8% died, none had liver lesions or ascites. Of the mice which received 5.0 mg cortisone, an average of 17% died, 9% had liver lesions, and 5% had ascites. Livers with cortisone-induced lesions were not infective for normal suckling mice. Several forms of unidentified bacteria were cultured in thioglycollate broth from these livers. Similar observations were reported by

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