+ P. As can be seen, the mean DNA of the mammary glands was reduced from 6.09 mg/100 g BW after EB + P administration to 3.59 mg after 20 days of testosterone (3 mg/day), which is the mean DNA of the gonadectomized rats (3.59 mg) prior to ovarian hormone treatment. It is clear that testosterone at the level of 3 mg/day is without value in preventing the involution of the lobule-alveolar system of male rats.

Summary. Adult male rats were gonadectomized for 20 days. The mean DNA of their mammary glands was 3.59 ± 0.27 mg/100 g BW. Similar rats were injected for 20 days with 2 mg EB + 6 mg P/day. The mean DNA of their glands was 6.09 ± 0.33 mg/100 g BW, a significant increase over the control group and equal to the level of DNA induced by the same ovarian hormones in overiectomized female rats. A similar group of gonadectomized rats treated with EB + P for 20 days was then injected with 3 mg testosterone/day for 20 days. Testosterone was ineffective in preventing involution of the lobule-alveolar system of these animals as indicated by the mean DNA of 3.59 ± 0.004 mg/100 g BW, which is the same as the group prior to EB + P treatment.

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Comparative Development of Pheasant and Chick Embryo Sera.* (31106)

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Although the serum of the chick embryo has been intensively studied(1,2,3,4), there are no reports concerning serum analysis of other avian embryonic sera, as far as can be ascertained.

Methods. Identical procedures were used to bleed 132 ring-neck pheasant embryos (*Phasianus colchicus*) and 325 chick embryos: the egg was opened at the air sac end with the inner shell layer carefully removed from the underlying chorioallantoic membrane. A large chorioallantoic artery was placed over a plastic trough secured over the shell opening. Adhering membranes were stripped away from the blood vessel. Blood was collected in chilled tubes, allowed to clot and centrifuged at 2000 × *g* for 30 minutes; the serum was stored at −20°C.

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Zone paper electrophoresis was performed in a horizontal apparatus utilizing Veronal-acetate buffer, $\mu = 0.1$, pH 8.6. Fifty μl of sera were applied to the paper strips followed by a current of 1.6 mA/cm strip width for 19 hours at 20°C(4). Densitometric tracings of the electropherograms, stained with aqueous bromphenol blue, were made with an Analytrol®.

Results. Fig. 1 illustrates the electropherograms of pheasant and chick embryo sera, respectively; while Table I shows the relative per cent concentration of serum protein components from the 10th to 21st days of incubation. Conventional electrophoretic terminology was used to name the respective serum fractions. A special component, S or sorbed continuum, was included in the computation of the relative per cent concentration. The

sorbed area comprised the region from the application line to the slowest resolved protein band.

Gross examination of Fig. 1 reveals a common increment as development proceeds in the amount of total serum protein in both

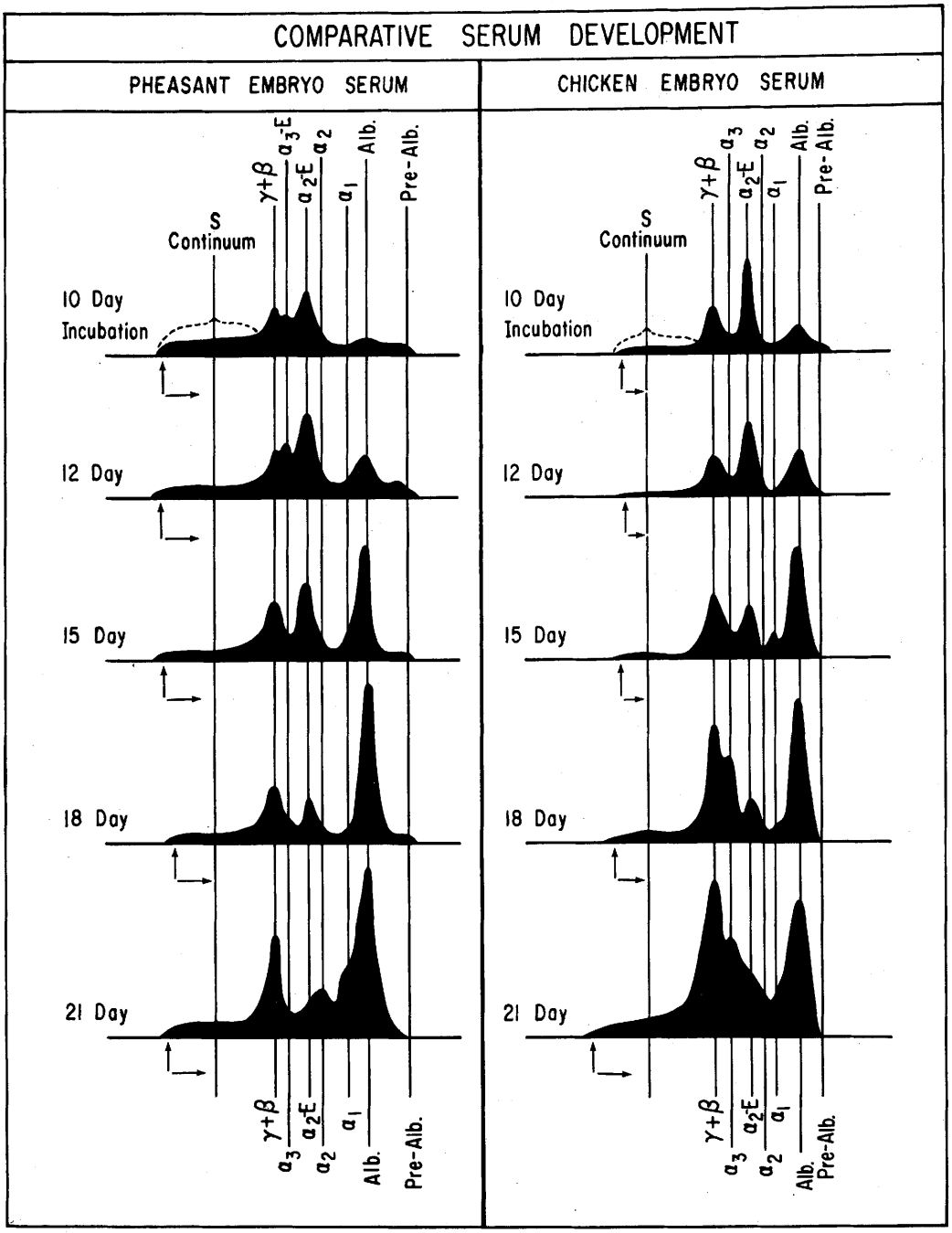

FIG. 1. Comparison of pheasant and chick embryo serum electropherograms. Vertical lines through serum components of identical electrophoretic mobility. Note alpha-3 globulin-E of pheasant embryo sera and alpha-3 globulin of chick have same mobility. Vertical arrows indicate application point; horizontal arrows show direction of migration of components towards anode.

TABLE I. Comparison of Pheasant and Chick Embryo Sera.*

Embryo species	Serum protein component(s)	Days incubation: 10 day	12 day	15 day	18 day	21 day
Pheasant	S	23.5‡ ± 7.1 (33)§	20.7 ± 6.3 (28)	19.1 ± 5.8 (29)	11.1 ± 3.2 (24)	7.5 ± 2.4 (18)
Chick	Continuum†	19.6 ± 6.6 (74)	18.5 ± 6.5 (66)	15.1 ± 4.4 (65)	10.4 ± 2.8 (96)	8.7 ± 2.7 (24)
Pheasant	Alpha and beta	13.6 ± 1.5	12.3 ± 1.3	23.0 ± 2.7	19.3 ± 2.4	20.1 ± 1.8
Chick	Globulins	23.1 ± 2.1	20.0 ± 1.6	22.8 ± 2.2	25.7 ± 1.9	33.8 ± 2.1
Pheasant	Alpha-3	—	—	—	—	—
Chick	Globulin	—	—	—	4.1 ± .2	7.2 ± .5
Pheasant	Alpha-3	16.2 ± 1.8	11.5 ± .9	—	—	—
Chick	Globulin-E	—	—	—	—	—
Pheasant	Alpha-2	27.0 ± 2.1	29.0 ± 3.0	24.1 ± 2.6	19.4 ± 2.2	10.5 ± 1.3
Chick	Globulin-E	40.6 ± 5.4	35.9 ± 2.5	21.1 ± 3.8	9.3 ± 1.8	—
Pheasant	Alpha-2	—	—	—	—	—
Chick	Globulin	—	—	—	—	6.4 ± .7
Pheasant	Alpha-1	—	—	—	—	16.7 ± 3.1
Chick	Globulin	—	—	4.8 ± 1.5	3.3 ± 1.9	15.6 ± 1.5
Pheasant	Albumin	10.5 ± 1.6	15.8 ± 2.3	35.5 ± 2.7	42.0 ± 3.1	45.6 ± 2.6
Chick	Albumin	13.9 ± 2.1	25.6 ± 4.5	36.7 ± 2.3	47.4 ± 2.7	38.8 ± 2.4
Pheasant	Pre-	12.0 ± 1.3	11.5 ± .8	8.3 ± 2.9	8.8 ± 2.5	—
Chick	albumin	3.4	—	—	—	—

* Relative per cent concentration.

† Components arranged according to electrophoretic mobility (fastest at bottom of table).

‡ Values represent means and standard deviations.

§ Number of animals.

species of embryonic sera, as indicated by the size (height and breadth) of most of the components (peaks). An increase in the complexity of the sera is also observed, reflected by an increase in the number of resolved electrophoretic serum fractions.

As Table I shows, similar quantitative changes, differing only in degree, were observed in the serum patterns of pheasant and chick embryo sera. Among these changes were: 1) a marked increase in albumin (especially in the period between 10 to 18 days incubation); 2) the presence of an embryo-specific alpha-2 globulin-E (-E, referring to embryo-specificity); 3) a similar marked decrease in both pheasant and chick embryo sera of alpha-2 globulin-E as development proceeded; 4) the slowest resolved group (gamma plus beta globulin complex) as well as the S continuum displayed parallel developmental changes with respect to relative concentration.

On the other hand, a unique feature of pheasant embryo serum was the presence (in 10- and 12-day old sera only) of an additional embryo-specific protein: alpha-3 globulin-E. Its highly acidic nature was indicated by the brilliant yellow-green color of aqueous bromphenol blue employed to dye the fractions. No similar staining protein was found in the sera from older pheasant embryos nor in sera from adult pheasants. A minor alpha-3 globulin appears in late chick embryo sera but without the staining qualities of the analogous pheasant embryonic serum protein.

Discussion. Strict chronological comparison of the sera of the 2 avian species of embryos was limited by the fact that the chick hatches at about 21 days incubation while the pheasant emerges from the egg at approximately 24 days incubation. Thus a 15-day pheasant embryo is developmentally comparable to a 12-day chick embryo. Nevertheless, chronological, not developmental criteria, were used to compare the sera from both species.

The similarities between pheasant and chick embryo sera were not unexpected because of the close taxonomic relationship between the pheasant (*Phasianus colchicus*) and the chicken (*Gallus domesticus*): both are in the Galliformes order. The prominence of alpha-2 globulin-E followed by its rapid decline in both pheasant and chick embryo sera suggests a similar function played by this protein in both species of embryos. Species differences are seen, however; alpha-2 globulin-E is still present as a minor component in 21-day pheasant embryo sera but has disappeared by hatching time in the chick. Recent immuno-electrophoretic studies pinpoint the disappearance of this component about one week after the chick hatches(5). The site(s) of synthesis of any of the serum proteins in either embryo is not known with certainty.

The emergence of albumin as the predominant serum protein in both species of embryos as well as its prodigious increase in concentration during the latter half of incubation, suggests similar synthetic mechanisms are operative. The uniqueness of alpha-3 globulin-E in younger stages of pheasant serum is exemplified by the fact that it is not seen in later pheasant embryonic or adult sera. Its abrupt disappearance after the 12th day of incubation suggests repression of synthesis.

The identity of the respective serum components resolved with paper electrophoresis may be modified with the use of more sensitive separation techniques such as disc electrophoresis. Alpha-3 globulin-E, for example, may be a beta rather than an alpha globulin. Thus the nomenclature adopted in this study to identify the serum fractions should not be interpreted in a strict literal sense.

On the basis of this study, analysis of other avian embryonic sera is suggested, with the possibility of seeing interspecies relationships apparent in the embryonic stage, but which disappear by the time the adult period of development is attained. In this connection, there is an interesting recent report of immunological similarity of fetal-specific globulins of the dog and man(6).

Summary. Pheasant and chick embryonic sera share common features as the embryo matures: emergence of albumin from a minor to a dominant position in the serum spectrum; the presence of an embryo-specific alpha-2 globulin-E which declines from a prominent position in the serum at mid-

incubation to either disappear (chick) or become a minor component (pheasant) by the 21st day of incubation. The pheasant embryo serum contains a stage-specific serum protein, alpha-3 globulin-E, which in addition is species-specific, *i.e.*, no similar staining protein is found in the chick embryo serum.

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Possible Role of Protein Binding in Failure of Antibody to Porcine Relaxin to React with Pregnant Rabbit Serum Relaxin. (31107)

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Noall and Frieden(1) reported that extended treatment of guinea pigs with porcine relaxin results in a decreased responsiveness to the hormone. Experiments designed to explain this phenomenon by immunological mechanisms were unsuccessful. Subsequent reexamination of the problem(2) led Frieden to postulate the probable formation of a species specific antibody to porcine relaxin since guinea pigs made resistant to porcine relaxin were capable of reacting to pregnant rabbit serum relaxin.

Cohen(3) described the preparation and biological properties of an antiserum to porcine relaxin and presented evidence indicating the absence of species specificity. Steinetz *et al*(4) extended and confirmed these observations but also noted the inability of antisera formed to porcine relaxin to neutralize the relaxin activity of pregnant rabbit serum.

Presented below are the results of our efforts to explain this anomolous behavior of circulating rabbit relaxin toward exogenous antibody to porcine relaxin.

Our experiments were based on the speculation that circulating rabbit relaxin is complexed with a serum component which does not inactivate the biological activity of the hormone but offers steric hindrance to the combination of the hormone with neutralizing antibody. If the foregoing conjecture is correct, the treatment of pregnant rabbit serum with agents which tend to dissociate such protein-protein combinations should free the serum relaxin from its conjugate and make it available for combination with antibody.

Materials and methods. 8 M urea and glacial acetic acid were used as dissociating agents.

132 ml of serum collected from female rabbits during the fourth week of proven pregnancy were lyophilized, yielding 7.5 g of dried powder.

a. 5 g of the above powder were suspended in 100 ml of glacial acetic acid, brought to 70°C and held at that temperature for 15 minutes. The solution was rapidly cooled, filtered and the hormone precipitated by addition of 4 volumes of acetone. The resulting precipitate was collected by filtration and dried *in vacuo* to yield 25 mg of powder

b. 2.5 g of the lyophilized pregnant rabbit serum was dissolved in 100 ml of 8 M urea at pH 7.8 and kept at room temperature for 5 hours. The solution was then precipitated with 10 volumes of acetone, the precipitate washed with acetone and dried *in vacuo*.

Antibody to porcine relaxin was prepared by the procedure described previously (Cohen. 3).