neural tissue could be found in the cranio-dorsal part of some embryos (Fig. 2*A*). Caudally, a large mass of poorly differentiated endoderm was frequently found. The mesodermal tissue in this region consisted almost exclusively of an extensive accumulation of renal tubules (Fig. 2*B*).

If the highly active fractions of the mesodermal factor were diluted by mixing with an inert protein (γ-globulin or albumin) and then implanted in the blastocoel of early gastrulae, they induced large mesodermal masses consisting of a notochord, surrounded by muscle, and of renal tubules (Fig. 3). In the isolated ectoderm ["sandwich" experiment (*10*)] both the undiluted and diluted fractions induced renal tubules, muscle, and notochord.

The exovagination effect may be explained as follows. Almost the entire ectoderm is induced to form mesoderm by the highly purified mesodermal factor; some of these ectodermal cells accumulate in the ventral, especially the ventro-caudal, part of the embryo. The epibolic movement of the ectoderm in dorsal and longitudinal directions fails to occur, as was revealed by vital staining of the surface (*11*); consequently, there is enlargement of the blastopore and reappearance of the yolk-rich endoderm. The most important change appears to be the change in cell affinities: all ectodermal cells induced to form mesoderm acquire an affinity for endodermal cells stronger than the initial affinity between endodermal cells; thus results the migration and spreading of endodermal epithelium over the ectoderm. The molecular changes in the cell-surface membranes responsible for this change in affinities are still unknown.

The experiments of Holtfreter *et al.* (*12*) demonstrate that tissue fragments isolated from gastrulae and neurulae, as well as single cells from dissociated tissue fragments, show marked preferences in their adhesive qualities. Mesodermal cells adhere to endodermal cells, with endoderm always surrounding mesoderm, whereas ectodermal and endodermal cells have no mutual affinities.

The results reported here are evidence that one of the first observable events initiated by the mesodermal factor in ectodermal cells is an alteration of cell affinities. Differential cell affinities and interactions must play an important role not only in cell movements but also in the further elaboration of different cell types. It remains to be determined how changes in cell surfaces are related to the intracellular control mechanism in the cell.

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7. Gastrulation, and the exovagination of the endoderm occurring thereafter, were followed after marks were set with Nile Blue sulfate and neutral red in the endodermal area of early gastrula stages. The migration of the endoderm depended on the species used in the experiment and was somewhat faster with *Triturus alpestris* than with *T. taeniatus*.
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9. Presumably, the mesodermal tissues found in these embryos were in part derived from the archenteron roof and in part induced; proportions of the two parts could only be determined in chimeric gastrulae obtained by heteroplastic transplantation of ectoderm.
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11. The marks were set in the early gastrula stage.
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Arbovirus Hemagglutinin Inhibitor in Acetone-Extracted Serums from Normal Chickens

Abstract. *A highly selective hemagglutinin inhibitor in serums from adult female chickens appears to be activated during extraction of the serum with acetone for removal of the usual lipid inhibitors. The acetone-insoluble inhibitor may be removed by filtration of the serum through Seitz pads. The precursor of the inhibitor in raw serum can be precipitated by two methods of removing euglobulins from solution.*

Relatively little is known about the normal serum inhibitors of arbovirus hemagglutinins. However, the fact that they are usually soluble in acetone and may be removed by adsorption on particles of kaolin or bentonite indicates that they may be lipids or lipoproteins. Reporting on studies with human serum, Salminen (*1*) concluded that four arboviruses of group A and six of group B shared the same nonspecific inhibitors. He further stated, "All these viruses were equally sensitive to the inhibitors." We are now reporting a selective inhibitor in adult female chicken serum, one which is variable in its effects on group A arboviruses and which causes no inhibition of the two strains of St. Louis encephalitis virus used in our tests. This inhibitor seems to be detectable only when the serum is first treated with acetone for the removal of lipid inhibitors.

Acetone precipitation and extraction of serum has been considered the reference procedure for removing normal inhibitors of hemagglutinins prepared from arboviruses (*2, 3*). In our usual procedure, 0.1 ml of serum is first diluted with 0.3 ml of 0.9 percent NaCl in water. The serum proteins are then precipitated and extracted with 6.0 ml of reagent grade acetone which has not been used previously. The precipitate is separated and extracted twice more with 6.0 ml of fresh acetone. The first extraction step is performed as rapidly as possible at 2° to 4°C. The following two steps require 10 minutes each and are carried out at room temperature. After extraction, the acetone insoluble precipitate is thoroughly dried and then dissolved in 1.0 ml of borate saline at *p*H 9.0. The resulting 1 : 10 serum dilution and subsequent twofold serial dilutions in borate saline are used as serum components in the standard hemagglutination-inhibition test. Presumably to minimize the denaturing action of acetone on serum proteins, Clarke and Casals (*3*) suggested ice-bath temperatures for the entire extraction pro-

Table 1. Arbovirus hemagglutination-inhibition titers of acetone-treated serums from 11 normal chickens. Titers are expressed as the highest serum dilution causing inhibition of eight units of the respective hemagglutinin. Zeros indicate no inhibition at the lowest tested serum dilution, 1:20. None inhibited hemagglutinins prepared with western virus strains 1703, 848, and B-11; two St. Louis strains (Parton and Webster); and one Venezuelan encephalomyelitis strain (designated Fort Detrick inactivated hemagglutinin).

Eastern encephalitis		Western encephalitis				
498	167	Olitsky	Fleming	85	McMillan	$L_2 34_a$
320	640	160	40	0	0	0
2560	2560	640	320	0	0	0
2560	1280	1280	320	160	40	40
640	640	320	40	40	0	0
1280	640	320	0	0	0	0
1280	1280	320	0	0	0	0
2560	1280	1280	0	0	0	0
5120	2560	1280	320	40	0	0
5120	2560	2560	640	80	80	80
2560	2560	1280	640	40	80	40
40	40	0	0	0	0	0

cedure. However, our experience has indicated that higher temperatures are needed to extract adequately the lipid inhibitors to which hemagglutinins of St. Louis encephalitis and eastern encephalitis viruses are unusually sensitive. With the above method we have successfully used acetone to remove normal inhibitors from serums of humans, horses, and smaller mammals.

Our first problem with nonspecific inhibitors was encountered (in 1960) when chicken serums were tested for hemagglutination-inhibition of an antigen prepared from the Olitsky strain of western encephalitis virus. Female chickens, hatched in the spring of the year and tested in the fall, had positive titers often exceeding 1 : 2560. Although the hemagglutination-inhibition titers remained the same after heating the serums to 56°C for 30 minutes, the presence of antibodies could not be confirmed by neutralization tests against the same strain of western virus (Olitsky). In roosters, the rate of positive hemagglutination-inhibition titers was strikingly lower than in female chickens. To illustrate, of 422 female chickens used as sentinels to detect arbovirus transmissions during 1959 in the state of Washington, 50 percent had high inhibitory titers against hemagglutinins prepared from Olitsky strain of western virus; yet only 5 percent of 416 mature roosters from the same flocks showed positive titers. Less than 1 percent of the serums from either roosters or hens inhibited hemagglutination by St. Louis viral antigens. These results stimulated studies on which this report is based.

Serums from more than 150 normal chickens were tested for the new inhibitor. Hemagglutinins prepared with the Olitsky strain of western virus were inhibited by nearly all acetone-treated serums from sexually mature hens. In these tests, inhibitory titers usually exceeded 1 : 320 and occasionally reached 1 : 5120. The inhibitor was not demonstrated in newly hatched chicks of either sex, in females before sexual maturity, nor in roosters of any age. None of the acetone-treated serums, at least in dilutions greater than 1 : 20, inhibited hemagglutination by St. Louis viral hemagglutinins (Parton strain). However, when the serums were merely diluted with borate saline, and not given prior treatment with acetone, fewer than 50 percent inhibited the Olitsky strain of western virus. When inhibition occurred, nearly all titers were 1 : 20, a few were 1 : 40, and none exceeded 1 : 80. Furthermore, the positive titers were distributed uniformly among chickens from all age groups and both sexes. Almost all of the raw serums inhibited St. Louis virus in serum dilutions ranging from 1 : 40 to 1 : 320. These results indicated that the acetone-insoluble inhibitor, at least in amounts detectable with hemagglutinins prepared from the Olitsky strain of western virus, was not transferred to progeny through the egg, that it developed only in female chickens, and that its appearance coincided with sexual maturity. Relatively small amounts of acetone soluble inhibitor were present in the untreated serums from chickens, regardless of age or sex. However, in serums of adult females, these inhibitors were either magnified greatly or, more plausibly, were replaced entirely by the acetone-insoluble factor which was active in much higher titers.

Further investigation revealed the following: (i) either the inhibitor in acetone-treated serum or the precursor in once-frozen raw serum could be removed by filtration through Seitz EK pads; (ii) the precursor of the inhibitor was precipitated by methods intended to remove euglobulins from solution. In these experiments, serum was diluted 1 : 10 in distilled water at 1° to 2°C and adjusted to *p*H 5.6 to 6.0 with dilute acetic acid or by overlaying with CO_2 gas; (iii) the inhibitor exerted its effect on the viral hemagglutinins, not on the goose red cells used in the test; and (iv) the inhibitor was formed during the first rapid step of acetone precipitation and extraction of chicken serum.

Finally, a comparison was made of the inhibitory effects of normal chicken serums on hemagglutinins prepared from several Group A and Group B arboviruses. We selected 11 adult females, 10 of whose serums had been found previously to inhibit hemagglutination by the Olitsky strain of western virus. Serum (0.5 ml) from each chicken was extracted with acetone, and portions were tested for inhibition of hemagglutinins of eight strains of western, two of eastern, two of St. Louis, and one of Venezuelan virus (Table 1). The marked selectivity of the inhibitor as shown in this experiment has been demonstrated repeatedly in hemagglutination-inhibition tests on serums from other normal chickens. Sensitivity to the inhibitor appeared to vary directly with the number of passages of the western virus strains through mouse brain. A similar relation was not shown with the two eastern strains.

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