bibe the liquid unless it contains appropriate stimulants. In our experiments, about 80 percent of the nymphs that had been starved for 2 years fed within 2 hours through such a membrane on sheep blood. Almost the same response was obtained when whole blood was substituted by a hemolysate of washed erythrocytes. No feeding took place on water or saline isotonic with blood.

The factor which stimulates the feeding response in the blood-sucking mosquito *Aedes aegypti* L. is adenosine triphosphate (ATP) (*1*). However, $10^{-2}M$ or $10^{-3}M$ ATP with or without the addition of $0.15M$ NaCl was refused by the ticks, and another stimulant had to be sought.

The red cell hemolysate was not utilized by the ticks after it had been subjected to extensive dialysis. Furthermore, when the hemolysate was dialyzed against an equal volume of saline for 48 hours, the diffusate was readily taken up by the ticks. The diffusate lost most of its activity after heating to 100°C for 15 minutes. Thus we inferred that a thermolabile compound of low molecular weight is at least partly responsible for the feeding response. Among the various red-cell constituents which we considered to have suitable properties, the tripeptide glutathione was chosen as a likely candidate. When glutathione (*2*) at a concentration of $10^{-2}M$ in saline, *p*H 7, was offered to the ticks, it was engorged rapidly. As a typical example, a 5-mg nymph imbibed 30 mg of this fluid in 10 minutes. To ascertain that the effect of the reduced glutathione was not due to an impurity we conducted the following test. To the solution of glutathione a slight excess of 5,5′-dithiobis (2-nitrobenzoic) acid was added. This compound combines specifically with sulfhydryl groups (*3*). After this treatment no feeding took place until addition of excess glutathione restored its original concentration. Table 1 shows that a significant response could be elicited also by a $10^{-3}M$ glutathione solution, but not by a $10^{-4}M$ solution. The concentration of glutathione in mammalian blood is of the order of $10^{-3}M$ (*4*). Thus the decreased response of the ticks to this concentration might indicate that other substances present in blood have an additive or sinergistic effect.

Table 1. The feeding response of *O. tholozani*. Abbreviations: GSH, reduced glutathione.

Compound	No. of experiments	No. of ticks	No. feeding (%)
Whole blood	5	139	78
Hemolyzed red cells	6	131	59
Distilled water	2	40	0
$0.15M$ NaCl	3	100	0
$0.3M$ sucrose	3	60	0
$10^{-2}M$ GSH*	2	40	5
$10^{-2}M$ GSH†	2	40	85
$10^{-2}M$ GSH‡	12	266	64
$10^{-3}M$ GSH‡	3	60	22
$10^{-4}M$ GSH‡	2	40	0
$10^{-2}M$ GSH‖	2	40	43

* In water. † In $0.3M$ sucrose. ‡ In $0.15M$ NaCl. ‖ In $0.15M$ KCl.

Table 2. Effect of glutathione analogues and some related amino acids on the GSH feeding response. Results expressed as the percentage of the total number feeding on the compound; $10^{-2}M$ GSH was added to each compound. Abbreviations: GSH, reduced glutathione; GSSG, oxidized glutathione; GABA, γ-amino butyric acid.

Compound*	No. feeding (%)
Control (GSH alone)	64 ± 3.6†
L-Glutamic acid	22 ± 5.4
D-Glutamic acid	28 ± 7.3
Glutamine	52 ± 6.9
GABA	56 ± 4.6
l-cysteine	59 ± 5.1
S-methyl-glutathione	60 ± 6.7
GSSG	71 ± 4.5
Glycine	71 ± 5.1

* All analogues were tested at $10^{-2}M$. † Standard error of the mean, calculated on the basis of results with at least 6 groups of 20 ticks each.

The tonicity of the solution was of paramount importance. Optimal results were obtained with solutions whose osmotic pressure was isotonic with that of blood. The nature of the solute did not seem to play a decisive role since both the nonelectrolyte sucrose and NaCl induced a similar response, while KCl was less effective. On the other hand, the response to glutathione was highly specific. No feeding response was obtained with oxidized glutathione, *S*-methyl-glutathione (*5*), or with any of the constituent amino acids: glycine, cysteine, or glutamic acid. Only glutamic acid, in either the L or D configuration, exerted considerable inhibitory action when used at concentrations equal to that of the glutathione present (Table 2). Since neither glutamine nor γ-amino-butyric acid produced significant antagonistic action, one can deduce that the glutamic acid moiety plays an important and specific role in the attachment of glutathione to its chemoreceptor site.

Other ticks, the common fowl tick *Argas persicus* (Oken) and the eyeless tampan *Ornithodoros moubata* (Murray), were also induced to feed by glutathione, but unlike *O. tholozani* a definite though small percentage of these populations also imbibed saline alone.

The specificity of glutathione in activating the sucking response of the tick bears a striking resemblance to the role of this tripeptide in the well-known case of the feeding reflex of *Hydra littoralis* (*6*). This is further emphasized by the inhibition exerted by glutamic acid (*7*). Although the sulfhydryl group (SH) of glutathione is not essential for its action on the *Hydra* receptor (*8*), blocking the SH by the methyl group renders the tripeptide ineffective as a stimulator of feeding for ticks. However, it is also possible that, as in the case of *Hydra* (*9*), other substances not related to glutathione will be found which have similar effect. In any case, our investigation may widen further the evolutionary significance of glutathione-activated receptors beyond the hydrozoan group (*10*).

RACHEL GALUN
S. H. KINDLER
Israel Institute for Biological Research, Ness-Ziona, Israel

References and Notes

1. T. Hosoi, *Nature* **181**, 1664 (1958); R. Galun, Y. Avi-Dor, M. Bar-Zeev, *Science* **142**, 1674 (1963).
2. Schwarz, BioResearch, Inc., New York, N.Y.
3. G. E. Ellman, *Arch. Biochem. Biophys.* **82**, 70 (1959).
4. E. C. Albritton, *Standard Values in Blood* (Saunders, Philadelphia, 1952), p. 108.
5. Synthesized by Zion Chemicals, Yavne, Israel.
6. W. F. Loomis, *Ann. N.Y. Acad. Sci.* **62**, 209 (1955).
7. H. M. Lenhoff and J. Bovaird, *Nature* **189**, 486 (1961).
8. E. E. Cliffe and S. G. Waley, *ibid.* **182**, 804 (1958).
9. H. Forrest, *Biol. Bull.* **122**, 343 (1962).
10. H. M. Lenhoff and H. A. Schneiderman, *ibid.* **116**, 452 (1959).
11. The skillful technical assistance of Mrs. H. Yordan is gratefully acknowledged.

24 November 1964

Exovagination of Newt Endoderm: Cell Affinities Altered by the Mesodermal Inducing Factor

Abstract. *Implantation of a highly purified mesodermal inducing factor in the blastocoel of early gastrulae results in the spreading of endoderm over the ectoderm. The effect can be explained by a change in cell affinities in the ectoderm, initiated by the mesodermal factor.*

"A central problem of cytodifferentiation is the identification and characterization of control factors extrinsic to the cell and the elucidation of the mechanisms by which these impinge upon the cell's inner controls" (*1*).

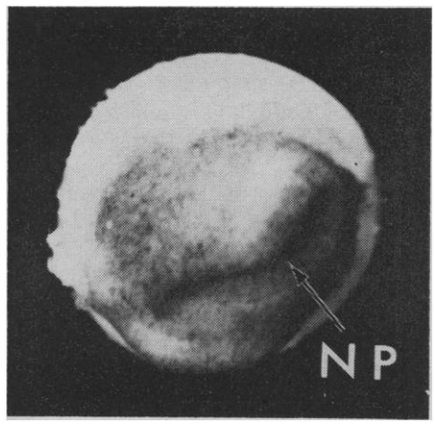

Fig. 1. A *Triturus* neurula, produced by treatment of early gastrula with highly purified mesodermal inducing factor, partially overspread by the migrating yolk-rich endoderm. A small neural plate is present (*NP*). (about × 30)

In one approach to the problem, we have attempted to isolate and characterize inducing factors from several sources. Factors capable of inducing mesodermal tissues, such as notochord, skeletal muscle, and renal tubules in competent ectoderm from *Triturus* gastrulae, have been extracted and enriched from chick embryos (*2*) and from guinea pig bone marrow (*3*). These factors are proteins, chemically related to those present in early amphibian embryos (*4*).

These inductors were tested by implanting pieces of washed, dried protein pellet of equal sizes in the blastocoel of early *Triturus* gastrulae (*5*). Preliminary experiments with the highly enriched mesodermal factor (*6*) revealed an unexpected effect. Initially, gastrulation—that is, invagination of the prospective endodermal and mesodermal areas—proceeded normally. However, after 14 to 18 hours of gastrulation, when the blastopore had closed or nearly closed, it "reopened", enlarging so that the bulk of the endoderm reappeared in the blastopore. Cells accumulated on the ventral side of the embryo, superficial to the implant, at least in part by migration of ectodermal cells. During the next 3 to 6 hours the endoderm migrated out of the blastopore and spread in a single layer over the ectoderm (*7*). With highly active fractions of the mesodermal factor, no neural plate was formed and the endoderm covered almost the entire embryo. If a fraction of moderate activity was used, the neural plate or part of it was formed, but the plate did not fold into a neural tube; in such embryos the endoderm spread over only a part of the ectoderm (Fig. 1). Further development of these embryos was extremely abnormal. After 14 days they had a more or less spherical form. No organs could be recognized externally, but it must be emphasized, that the embryos were fully viable. The process differed markedly from the exogastrulation described by Holtfreter (*8*).

Histological examination of these

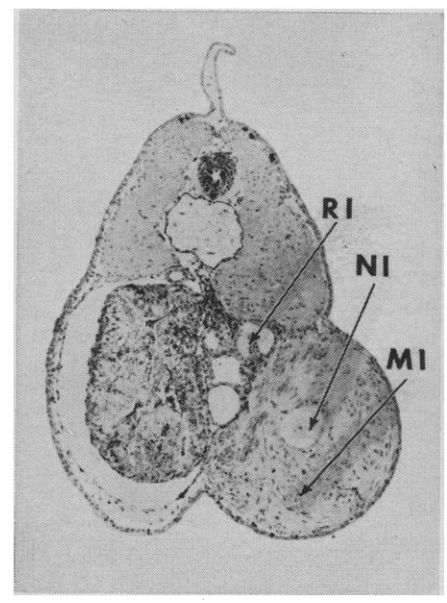

Fig. 3. Section through a 14-day-old *Triturus* embryo after treatment of the gastrula with a mesodermal inducing factor "diluted" with γ-globulin about 200 times. The large mesodermal induction on the right side of the embryo consists of notochord (*NI*), muscle (*MI*), and renal tubules (*RI*). (about × 40)

embryos showed them to consist almost entirely of tissues derived from mesoderm (*9*) and endoderm; the endodermal epithelium always surrounded skeletal muscle, notochord, and renal tubules. The embryos had no brain or neural tube, or any of the related organs. Only a little irregularly shaped

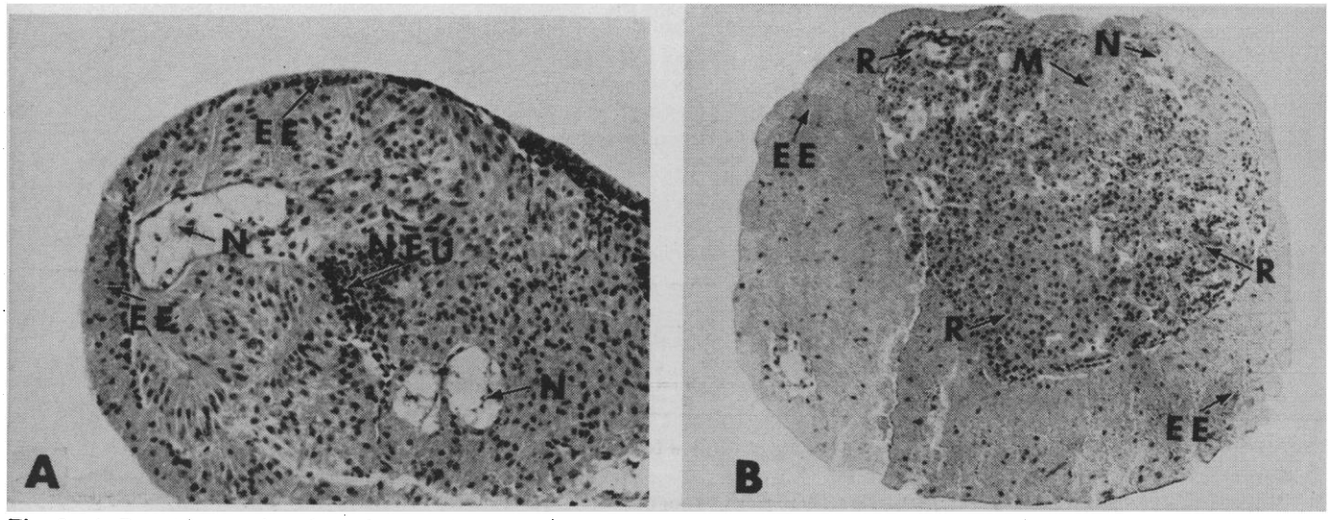

Fig. 2. *A*, Part of a section through a 14-day-old *Triturus* embryo which had been treated at the gastrula stage with highly purified mesodermal factor. Notochord and skeletal muscle are surrounded by endodermal epithelium (*EE*). Between the two pieces of notochord (*N*) is some irregular neural tissue (*NEU*). (about × 100) *B*, Section through the caudal part of a 14-day-old *Triturus* embryo after treatment of the gastrula with highly purified mesodermal inducing factor. Poorly differentiated endoderm (*EE*) surrounds the mesodermal tissue which forms a large number of renal tubules (*R*). Other abbreviations: *N*, notochord; *M*, muscle. (about × 40)

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.

URSULA KOCHER-BECKER
HEINZ TIEDEMANN
HILDEGARD TIEDEMANN
Carnegie Institution of Washington, Department of Embryology, Baltimore, Maryland, and *Heiligenberg-Institut, Heiligenberg/Baden, West Germany*

References and Notes

1. C. Grobstein, *Science* **143**, 643 (1964).
2. H. Tiedemann and H. Tiedemann, *Z. Physiol. Chem.* **314**, 156 (1959); H. Tiedemann, in *Biological Organization at the Cellular and Supercellular Level*, R. C. Harris, Ed. (Academic Press, New York, 1963), p. 183.
3. T. Yamada, *J. Cell. Comp. Physiol.* **60**, (suppl. 1), 49 (1962).
4. H. Tiedemann, in "Induktion und Morphogenese," 13. Mosbacher Coll. Ges. Physiol. Chemie (Springer, Berlin, 1963), p. 177.
5. O. Mangold, *Arch. Mikroskop. Anat.* **100**, 198 (1923).
6. The factor was enriched about 10,000 times; it was purified by chromatography on CM-cellulose in the presence of 6*M* urea and by zone electrophoresis. The molecular weight is about 25,000. The entire procedure will be published elsewhere. To handle the small quantities in the test experiments the factor was mixed with inert protein (γ-globulin, albumin) in different proportions.
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*.
8. J. Holtfreter, *Arch. Entwicklungsmech. Organ.* **129**, 669 (1933).
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.
10. J. Holtfreter, *Arch. Entwicklungsmech. Organ.* **128**, 584 (1933).
11. The marks were set in the early gastrula stage.
12. J. Holtfreter, *Arch. Exptl. Zellforsch.* **23**, 169 (193 → P. J. Townes and J. Holtfreter, *J. Exptl. Zool.* **128**, 53 (1955).
13. We thank Dr. J. D. Ebert for reading the manuscript.

13 November 1964

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-