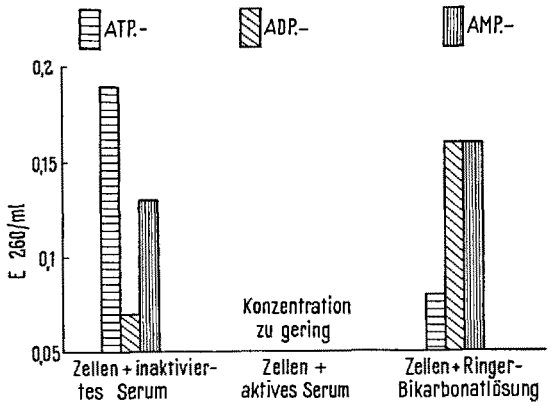

Einfluss von heterologem Aszitestumor-Antiserum und Komplement auf ATP, ADP und AMP von EAT-Zellen. Die erhaltenen Extinktionswerte lagen nicht mehr im Gültigkeitsbereich der Methode. Kontrollen: Bei 56°C 30 min erhitztes Antiserum und Ringer-Bikarbonatlösung. Reaktionsdauer 1 min bei 37°C. Auf der Ordinate ist die UV-Absorption bei 260 mμ der auf der Abszisse aufgetragenen Nukleotidfraktionen angegeben.

Es wurde daher das Verhalten der ATP der EAT-Zelle in Gegenwart von heterologem Antiserum und Komplement untersucht.

Material und Methoden. Alter des EAT, aktives und inaktives Immunserum wie bei [2]. Bestimmung der Nukleotide: Papierchromatographische Trennung der Adenosintriphosphorsäure, Adenosindiphosphorsäure (ADP) und Adenosinmonophosphorsäure (AMP), Photoprintverfahren, Elution der Flecken mit $n/10\ M$ HCl und Messung der relativen Nukleotidkonzentrationen im Beckman-Spektrophotometer. Zu jedem Experiment mit komplementhaltigem Serum wurden zwei Kontrollen angesetzt, eine mit Serum, dessen Komplementaktivität bei 56°C in 30 min zerstört wurde, und eine Kontrolle in Ringer-Bikarbonatlösung. Ansatz: 3,5 ml aktives Serum, inaktiviertes Serum bzw. Ringer-Bikarbonatlösung + 0,5 ml Zellsuspension des EAT 1 min bei 37°C geschüttelt, Medien wurden auf 37°C vorgewärmt. Zentrifugation in auf −10°C gekühlten Zentrifugeneinsätzen in der Kälte, Sedimente mit kalter Trichloressigsäure versetzt.

Ergebnisse. Die Figur zeigt, dass unter den Bedingungen der Immuncytolyse Zellen des EAT eine schnelle und relativ starke Reduktion ihres ATP-, ADP- und AMP-Gehaltes erfahren. Der Effekt ist an die Gegenwart von Komplement gebunden.

Es ist wahrscheinlich, dass der früher beschriebene Strukturverlust der Zelle nach kurzfristigem Einwirken von heterologem Antiserum und Komplement durch den Verlust der ATP bedingt ist[2]. Ferner erklärt der Schwund der ATP die Hemmung der Glykolyse, da ATP zur Phosphorylierung der Glukose notwendig ist. Der Verlust der Nukleotide kann auf zwei Wegen erfolgen, entweder durch Abbau oder durch Hemmung der Synthese von ATP, jedoch spricht der rapide Schwund der ATP für den ersteren Mechanismus. Es ist nicht ausgeschlossen, dass durch die Antigen-Antikörperreaktion in Gegenwart von Komplement eine fermentative Reaktion an der Zellmembran ausgelöst wird, die in Richtung auf das Innere der Zelle den ATP-Abbau bewirkt. Wie schon früher erläutert wurde[2], erfasst der kurzfristige Versuch mit verdünnten Antiseren Vorgänge an der Zelle, die später infolge Austritts von Zellinhalt durch die aufgelockerte Zellmembran nicht mehr zu untersuchen sind[2,3]. Die Experimente machen es wahrscheinlich, dass die Ursache der Immuncytolyse ein ungewöhnlich schnelles Verschwinden von energiereichem Phosphat (ATP) aus der Zelle ist.

Summary. Heterologous antibodies of the Ehrlich ascites tumor, in the presence of complement, cause a rapid drop of the ATP in the cells of this tumor. Probably there is a relation between this effect and the immune cytolysis.

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Lilienstrasse 7, Landstuhl/Pfalz (Deutschland), 28. September 1960.

[3] B. GOLDBERG and H. GREEN, J. exp. Med. *109*, 505 (1959). – H. GREEN, R. A. FLEISCHER, and B. GOLDBERG, J. exp. Med. *109*, 511 (1959).

[4] Fräulein MARIANNE KAESTNER und Frau CHRISTA DUBA danke ich für ihre gewissenhafte Mitarbeit. Die Untersuchungen wurden 1957–1959 am Max-Planck-Institut für Virusforschung in Tübingen und am Pathologischen Institut der Universität München, zum Teil mit Mitteln der Deutschen Forschungsgemeinschaft, durchgeführt.

Molecular Ontogenesis and Molecular Genetics in the Crosses of Toads

An investigation was carried out in order to study the appearance of antigens during the embryonic development of two toad species (*Bufo bufo* L. and *B. viridis* Laur.) and of their reciprocal crosses. It is well known that the *B. bufo* ♀ × *B. viridis* ♂ cross is viable, while the *B. viridis* ♀ × *B. bufo* ♂ cross is lethal: the latter hybrid dies during neurulation and only a few specimens (about 10%) survive and reach a more advanced stage of development.

Rabbits were immunized with powder of the lyophylized total body of *B. bufo* or of *B. viridis*. Bodies of adult male animals without testicles were used as 'total body'. The methods of preparation of the antisera and for testing the antigens in test tubes are described in the references[1,2]. We call here anti-bufo the rabbit antiserum prepared against *B. bufo*, and anti-viridis the antiserum prepared against *B. viridis*.

The egg extract does not precipitate with the antisera: the first precipitine reaction appears during the cleavage. Such a reaction is specific for the antigens of the same species. The precipitation in the hybrids is produced by the antisera of both the species. These results mean that substances are synthesized during cleavage under the influence of both paternal and maternal genes. The present experiments were not designed to test whether these substances are in the nucleus, the cytoplasm or both.

But the antigens of the crosses can also be studied to recognize some aspects of the action of genes on the developing molecules.

In the parental species, one antigen (or group of antigens) precipitating from a solution in Weber and Edsall fluid, at a concentration of ammonium sulphate between 30 and 50% of saturation, is species-specific at the early gastrula stage and, at the tail bud stage, it is also precipitated by the antiserum against the other species of *Bufo*.

When it is possible to precipitate the extracted proteins with the antisera prepared against the two species of toads, we tested the same sample first with one antiserum

[1] S. RANZI, P. CITTERIO, and C. SAMUELLI, Ist. Lombardo (Rend. Sci.) [B] *92*, 468 (1958).

[2] S. RANZI, P. CITTERIO, and C. SAMUELLI, Acta embryol. morph. exp. *3*, 65 (1960).

Fraction precipitating between 30 and 50% $(NH_4)_2SO_4$ saturation

	early gastrula stage 11		neurulation stage 14		tail bud stage stage 18
B × B	B	→	B	→	BV
V × V	V	→	V	→	BV
V × B	B	→	B	→	BV
B × V	B	→	B,V	→	BV

Fraction precipitating between 50 and 70% $(NH_4)_2SO_4$ saturation

	late blastula stage 9		early gastrula and neurulation stages 11 and 14		tail bud stage stage 18
B × B	B	→	B	→	BV
V × V	V	→	V	→	V
V × B	BV,V	→	BV,V	→	B,V
B × V	BV,V	→	BV	→	BV

Fraction precipitating between 70 and 90% $(NH_4)_2SO_4$ saturation present only since the tail bud stage

B × B	B
V × V	V
V × B	B,V
B × V	B,V

Results and interpretation:

B × B: *B. bufo;* V × V: *B. viridis;* V × B: *B. viridis* ♀ × *B. bufo* ♂; B × V: *B. bufo* ♀ × *B. viridis* ♂; the stages are those taken from the book by RUGH[3].

B = precipitation by the anti-bufo serum;

V = precipitation by the anti-viridis serum;

B,V = precipitation by anti-bufo serum, after precipitation by anti-viridis serum and viceversa (two different molecules);

BV = precipitation by anti-bufo and anti-viridis sera, but not two precipitations of the same sample (two reactive groups in the same molecule);

BV,V = precipitation by anti-viridis serum after precipitation by anti-bufo serum but not *viceversa* (possible interpretation: two kinds of molecules are present both with reactive groups for anti-viridis, but only one with reactive groups for anti-bufo).

and successively with the other one: in order to recognize whether the reactive groups are located in two different molecules. Two results are possible: 1. the extract, precipitated by the first antiserum, does not precipitate with the second one: the two reactive groups seem to be located in the same molecule; 2. the extract is precipitated by the first antiserum, and then also by the second one: two kinds of molecules seem to be present one for the first reactive group(s), the other for the second one(s).

Molecular ontogenesis. Antigens (or groups of antigens) are present in *B. bufo* embryo during gastrulation and neurulation: they are precipitated from a solution in Weber and Edsall fluid at a concentration of ammonium sulphate between 30 and 50% saturation and 50 and 70% saturation: these fractions are precipitated by anti-bufo serum, but they are not precipitated by anti-viridis serum. The precipitation by anti-viridis serum starts at the tail bud stage. It is impossible to precipitate these fractions by anti-viridis serum after precipitation by anti-bufo serum or by anti-bufo serum, when precipitated by anti-viridis serum. If we call B the molecules with a reactive group for anti-bufo serum, V the molecules with a reactive group for anti-viridis serum and BV the molecules with both reactive groups, we can assume that during gastrulation and neurulation B is present, while at the tail bud stage, B disappears and BV is present.

In the fraction, precipitating at a concentration of ammonium sulphate between 30 and 50% saturation, of the viable cross *B. bufo* ♀ × *B. viridis* ♂, a different ontogenesis of the antigens was discovered. At the early gastrula stage, only the antigens B were present. At the neurula stage, both antigens B and V were present. But at the tail bud stage, only BV antigens were present.

Molecular genetics. Reciprocal crosses were used to study the gene actions at a molecular level. In the fraction, precipitating at a concentration of ammonium sulphate between 50 and 70% saturation, of the viable cross *B. bufo* ♀ × *B. viridis* ♂ only antigens BV could be detected after the early gastrula stage, but these antigens were absent at the tail bud stage of the lethal cross *B. viridis* ♀ × *B. bufo* ♂. It is possible to detect in this cross at the tail bud stage antigens B and V represented by different molecules. We can, therefore, show evidence of a second characteristic of the lethal cross. The lethal cross is unable to combine the two reactive groups in the same molecule, whereas this possibility exists in the reciprocal cross. The other characteristic of the lethal cross is that some antigen(s) starts only with the paternal reactive group (antigen precipitating at a concentration of ammonium sulphate between 30 and 50% saturation, see the Table and [1,2]). The incapacity to combine the two reactive groups in the same molecule can be related to the low synthetic activities well known in the lethal crosses of the amphibians.

Riassunto. È stata studiata la comparsa di antigeni nel corso dello sviluppo di due specie di rospi: *Bufo bufo* e *B. viridis* e dei loro incroci reciproci. Alcuni antigeni al loro primo apparire precipitano solo con l'antisiero specie-specifico e, solo dopo lo stadio di bottone caudale, sono precipitati anche dall'antisiero dell'altra specie; questo viene interpretato quale una ontogenesi molecolare. Negli incroci, le molecole con gruppi reattivi per i due antisieri sono più frequenti nell'incrocio vitale (*B. bufo* ♀ × *B. viridis* ♂), mentre l'incrocio letale (*B. viridis* ♀ × *B. bufo* ♂) mostra alcuni antigeni separati che precipitano ognuno con un antisiero.

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Istituto di Zoologia, Università statale, Milano (Italy), October 7, 1960.

[3] R. RUGH, *Experimental Embryology* (Burgess, Minneapolis, Minn. 1952).

[4] This investigation was supported by a grant from the Consiglio Nazionale delle Richerche.

Electron Diffraction and Electron Microscopy Studies on the Crystalline Component of Enamel of the *Odontaspis* (Selachii)

The nature and structure of fish tooth enamel present many problems and some points as yet not well understood. Some authors[1,2] consider fish enamel as a true enamel, but others believe it is a modified form of dentin which they call 'vitrodentin'[3] or 'fibrodentin'[4]. Due, also, to the great interest from the histogenetic point of view[5],

[1] C. TOMES, *Traité d'anatomie dentaire – Humaine et comparée* (Octave Doin, Paris 1880), p. 88.

[2] T. W. WIDDOWSON, *Special or Dental Anatomy and Physiology and Dental Histology*, Vol. II, 7th Ed. (Staples Press Ltd., London 1946), p. 67.

[3] C. RÖSE, Anat. Anz. *14*, 33 (1898).

[4] J. J. THOMASSET, Arch. Anat. Histol. Embryol. *11*, 5 (1930).

[5] L. LISON, in *Traité de Zoologie* (Pierre P. Grassé), Vol. XII (Masson & Cie., Paris 1954), p. 791.