The Proteins in the Cells and in Embryonic Development[1]

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A viscosimetric analysis of the shape of protein particles

A viscosimetric method allowing one to recognize the shape of protein particles in solution has been devised. The method is based on the differences in behaviour of the different kinds of proteins upon denaturation[3].

Two samples are prepared from a protein solution. In the first, KSCN is added up to 0·05–0·1 m final concentration. In the second (the control) the same amount of KCl is added.

To 10 ml of protein solution (from 0·01% to 0·1%) 0·6 ml of 1 m KSCN are added. 10 ml of the same solution plus 0·6 ml of 1 m KCl are used as control[4]. After standing 4 hours at 10°–20° C (or overnight at 0°C) the viscosity was measured in an Ostwald viscosimeter. Owing to the low concentrations used in our experiments, determination of density is not necessary.

In a protein solution with fibrillar particles, the specific viscosity $\eta_{sp} = \frac{t_1}{t_0} - 1$ (where t_1 is the outflow time for the solution and t_0 is that of the solvent) of the sample treated with KSCN is lower than the one treated with KCl. On the other hand, in a protein solution with globular particles the specific viscosity of the KCl-treated control is lower than that of the sample treated with KSCN.

The values of specific viscosity of proteins of known molecular shape (by flow-birefringence, electron microscope, ultracentrifuge) are given in figure 1. It shows a good agreement between our data and those obtained with other methods.

The KCl and KSCN samples show also differences in surface tension. Using Du Noüy's tensiometer, solutions of fibrillar shaped proteins show a lower surface tension when treated with KSCN than when treated with KCl. On the other hand, solutions of globular proteins show higher surface tension when treated with KSCN than when treated with KCl. However, the differences in surface tension are very small, whereas those obtained by viscosity measurements are much greater.

The effect of the following salts was tried in parallel samples: KCl and KI; KCl and $KClO_3$; KCl and KBr; KCl and KNO_3; NaCl and NaSCN; NaCl and NaI; in the same way as reported for KCl and KSCN. It has been found that in the presence of KI, $KClO_3$, KBr, KNO_3, NaSCN, NaI the viscosity of solutions of fibrillar proteins is lowered with respect to that of the same proteins in KCl or NaCl. Solutions of globular proteins on the contrary, under the same conditions, show increase in viscosity. Such viscosity changes are induced by all swelling ions. The activity of these ions follows the Hofmeister's series $SCN > I > Br > ClO_3 > NO_3$. The decrease in viscosity of solutions of fibrillar proteins is rather conspicuous in presence of SCN, while it is small with NO_3.

SCN and I were used in the following experiments because of their stronger effect on viscosity.

An experiment (fig. 2) was carried out as follows: to an actomyosin solution in buffered 1 m KCl, constant amounts of 1 m KCl, or of a mixture of 1 m KCl + 1 m KSCN in different proportions, was added.

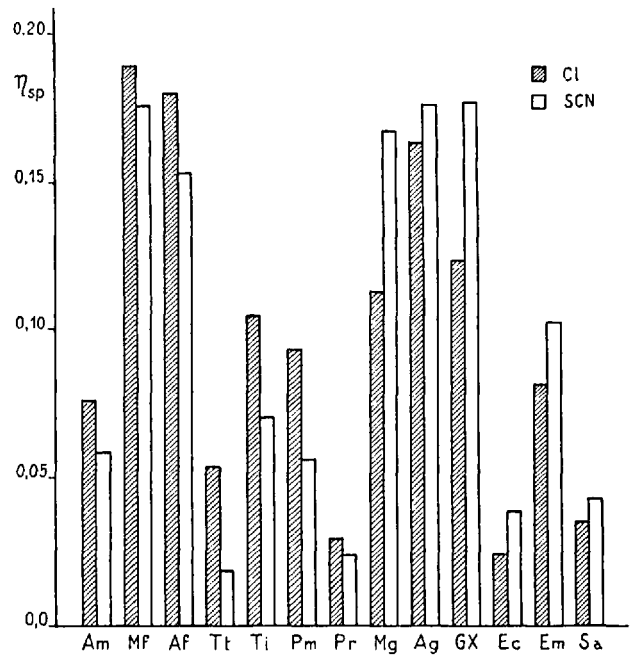

Fig. 1. – Specific viscosity of some protein solutions with the described method. The shaded column is the viscosity of the control (with KCl), the adjacent white column is the viscosity of the sample treated with KSCN. Am, pigeon actomyosin; Mg, Szent Györgyi's myosin of pigeon dissolved in 0·1 m KCl (fibrillar); Af, pigeon actin at p_H 6·5: Tt, sodium thymonucleinate from ox pancreas; Ti, thymonucleohistone from pigeon erythrocytes; Pm, Mirsky's protein from sea urchin eggs; Mg, Szent Györgyi's myosin of pigeon dissolved in 0·5 m KCl; Ag, pigeon actin at p_H 8·0; GX, globulin X of pigeon muscles; Ec, euglobulin C from sea urchin eggs; Em, pigeon hemoglobin; Sa, serum albumin. The proteins represented on the left side of diagram show fibrillar shaped particles; in all these proteins the viscosity of the control (with KCl) is the highest. The proteins represented on the right side show globular shaped particles; in all these proteins the viscosity of the sample treated with KSCN is highest.

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[3] S. Ranzi, Boll. Soc. Ital. Biol. Sper. *24*, 396 (1948). – M. Cigada, P. Citterio, A. Orlandi, S. Ranzi, and L. Tosi, Rend. Ist. Lombardo Sc. e Lett. (Cl. Sc.) *82*, 351 (1949).

[4] According to our experience an error of 0·5 ml over 10 ml is immaterial for the result. The same is true for an error of 0·05 ml over 0·6 ml.

The increase of KSCN concentration up to a final concentration of about 0·01 m induces a decrease in the viscosity. Higher concentrations of KSCN cause increase in viscosity sometimes up to the value of the control with KCl. The flow-birefringence decreases with the increase of KSCN concentration and falls to zero for the highest concentrations of KSCN.

KSCN, at least up to a concentration of 0·5 m, added in the same way to a solution of a globular protein (i. e. globulin X of the muscle or hemoglobin) causes an increase in viscosity (fig. 2).

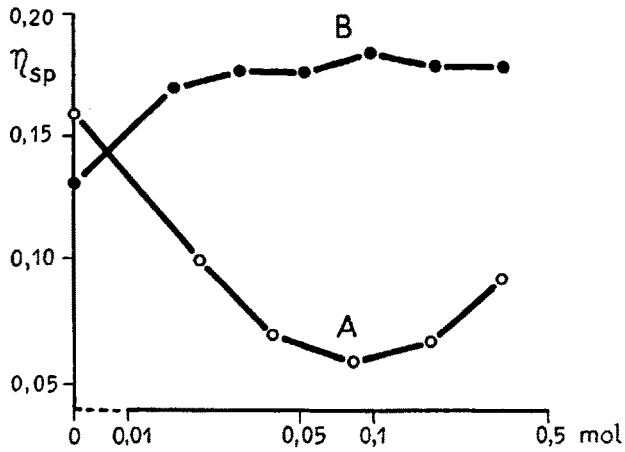

Fig. 2. – Specific viscosity on the ordinate of solutions A) of actomyosin; B) of globulin X of muscles; on the abscissa the concentration of KSCN substituted in the solvent for equimolecular amounts of KCl.

The behaviour of the two kinds of proteins for increasing concentrations of KSCN up to 0·1 m, as illustrated in the left part of the two diagrams, can be compared with the phenomena occurring on protein denaturation. On denaturation globular proteins show increase in viscosity: fibrillar proteins show decrease in viscosity[1].

With the electron microscope, actomyosin treated with KSCN (or KI) shows shorter particles. The same is true for thymonucleohistone. For the lower concentrations of KSCN, particles are short. At high concentrations of KSCN particles appear as globular. This change is irreversible. Actomyosin, globular after treatment with KSCN, was precipitated by dilution and redissolved. The operation was repeated a few times. Even after complete removal of KSCN from the solvent, the actomyosin appeared still as globular.

The increase in viscosity of a solution of a globular protein induced by KSCN can be due to a swelling of the protein particles. The increase of specific viscosity $\eta_{sp} = \nu\, \Phi$ (ν being a value related to the anisotropy of particles and Φ the portion of the solution occupied by protein particles), induced by KSCN, cannot depend on a variation of ν. In a solution of hemoglobin indeed the flow-birefringence does not appear. The increase of specific viscosity thus is to be accounted for by an increase of Φ, namely a swelling of protein particles induced by KSCN.

The increase in viscosity of a solution of a fibrillar protein for a further increase of KSCN concentration above 0·1 m parallels the increase observed in a solution of a globular protein in presence of KSCN.

Shape and structure of the protein particle

With the described viscosimetric method, globular proteins in solution, as e. g. hemoglobin, can be distinguished from the fibrillar ones, as e. g. actomyosin, even in such diluted solutions, for which analysis of flow-birefringence fails to give any result.

Besides, four kinds of proteins can be identified by comparison of the data obtained with this method and those obtained from flow-birefringence in GERANDAS' cell (table).

Protein shape	Shows higher viscosity with	Flow-birefringence	Example of protein
globular . . .	KSCN	absent	hemoglobin
fibrillar folded	KCl	absent	euglobulin a + b from egg
fibrillar . . .	KCl	present	actomyosin
sponge shaped	KSCN	present	rigid thymonucleohistone

The data, just referred to, show that fibrillar protein particles can be broken into smaller particles. Those appear as isodiametric with our methods. Fibrillar actin is built up according to this scheme[1]; p_H changes induce globular actin to polymerize into fibrillar actin and fibrillar actin to depolymerize. By varying p_H we succeeded in transforming actomyosin and $a + b$ fractions of euglobulin of frog and of sea urchin eggs into protein which appears as globular. $a + b$ globular euglobulin cannot be transformed into fibrillar particles, but that is possible with actomyosin.

It is possible that the fibrillar proteins are built up by polypeptide chains united by bonds which are broken by SCN or I. Once these bonds have been broken the smaller particles exhibit globular shape with our methods (decrease in viscosity, disappearance of the flow-birefringence). Further breaks induce greater disorder of the polypeptide chains and the particles become ramified (increase in viscosity without appearance of flow-birefringence).

At this point we thought that the chromosomes, isolated by MIRSKY and RIS' method[2] could be taken as a model of a fibrillar protein particle, on the assumption

[1] H. NEURATH, J. P. GREENSTEIN, F. W. PUTNAM, and J. O. ERICKSON Chem. Rev. *34*, 157 (1944).

[1] A. SZENT GYÖRGYI, *Chemistry of muscular contraction*, Academic Press, New York (1947).

[2] A. E. MIRSKY and H. RIS, J. gen. Physiol. *31*, 1 (1947).

that the bonds uniting the particles of chromosome structure were the same as those uniting the polypeptide chains in a fibrillar protein particle. The isolated chromosomes were treated with 0·16 m KSCN or KI or at high p_H. Chromosomes disintegrate in long threads. If we imagine a chromosome built in a similar way as a protein molecule, the break pattern of a chromosome appears to be similar to that of a fibrillar protein particle setting free polypeptic chains, when broken down.

Some proteins from animal tissues

Experiments were carried out, with our methods, using muscular proteins. These were considered a good object, as many of their properties have been largely investigated in recent years. The results have already been published in this Journal[1]. The electron microscope pictures have been published by CIGADA[2].

AROSIO and ORLANDI (unpublished data) also succeeded in extracting from the electric organ of Torpedo a small amount of a myosin-like protein. This latter gives also threads which contract with ATP. An actin-like protein can also be extracted from the electric organ; that can be combined with mouse myosin. A complex is thus obtained which is able to give threads.

Thymonucleohistone was also studied with our methods. As I already said, the sponge shaped particles of this substance show an increase in viscosity with SCN, but show also flow-birefringence. If thymonucleohistone is broken, mechanically or by dilution, flow-birefringence is still present but with SCN viscosity decreases as in all fibrillar proteins[3].

Embryonic proteins

The investigations of MIRSKY[4] and CONNORS and SCHEER[5] have shown the presence of a fibrillar protein in sea urchin egg extracts. This protein fraction is fibrillar shaped when prepared by precipitation with ammonium sulphate. If on the other hand precipitation is carried out by dilution, and the protein is redissolved in 1 m KCl, it shows fibrillar folded particles[6]. Two fractions can be separated from this protein: euglobulin *a* and euglobulin *b*. These two fractions are present in sea urchin, frog and fowl eggs. Other globulin fractions are present in all these eggs: euglobulin *c*, which shows globular shaped particles, and pseudoglobulin, which shows fibrillar folded particles.

Chicken embryos (Rhode Island Red) 6 days old contain large amounts of globular proteins soluble in 0·1 m KCl. From the 9th day on, a large synthesis of fibrillar or fibrillar folded proteins can be recognized. After the 16th day it is possible to demonstrate the synthesis of a large amount of proteins insoluble in 0·6 m KCl containing 30 per cent of urea. A part of this insoluble protein is keratin. The figures show that a transformation of globular proteins into fibrillar shaped ones and of fibrillar soluble proteins into insoluble ones is likely to occur.

During the development of the frog egg, the formation of the intercellular spaces and of the circulatory system is related to an increase of globular shaped proteins. These proteins arise from the fibrillar folded ones of the yolk.

Proteins and embryonic determination

Let us now take into consideration the behaviour of different kinds of proteins during embryonic determination. The existence of substances able to induce changes in embryonic determination (fig. 3) is known. LiCl

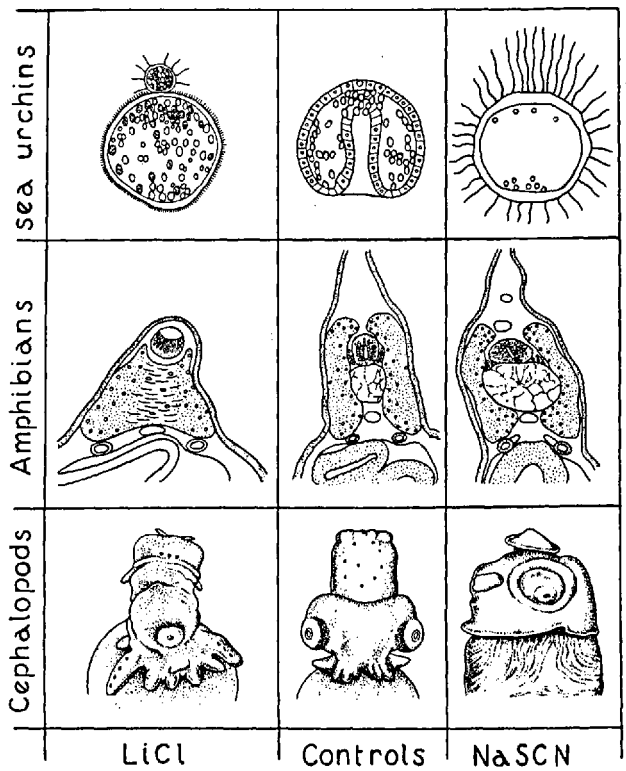

Fig. 3. – Scheme of the different changes induced by LiCl and NaSCN in the embryonic development: in sea urchins; in Amphibians (cross section of young tadpoles); in Cephalopods (embryo seen from the dorsal side). In the Cephalopods KSCN instead of NaSCN was used (from RANZI 1947).

induces an hyperdevelopment of entoderm and mesenchyme at the expense of ectoderm in sea urchin embryos (vegetalization)[1], reduction of notochord[2] and cyclopia[3] in Amphibians and Cyclostomes[4], cyclopic

[1] M. CIGADA, P. CITTERIO, S. RANZI, and L. TOSI, Exper. *4*, 480 (1948).
[2] M. CIGADA, Boll. Soc. ital. Biol. sper. *25*, 669 (1949).
[3] M. CIGADA, P. CITTERIO, A. ORLANDI, S. RANZI, and L. TOSI, Rend. Ist. Lombardo Sc. e Lett. (Cl. Sc.) *82*, 351 (1949).
[4] A. E. MIRSKY, Science *84*, 333 (1936).
[5] W. M. CONNORS and B. T. SCHEER, J. cell. comp. Physiol. *30*, 271 (1947).
[6] R. AROSIO, P. CITTERIO, S. RANZI, and L. TOSI, Rend. Ist. Lombardo Sc. e Lett. (Cl. Sc.) *82*, 163 (1949).

[1] J. RUNNSTRÖM, Roux'Arch. *113*, 556 (1928).
[2] F. E. LEHMANN, Roux'Arch. *138*, 106 (1938).
[3] G. CONTRONEI, Arch. ital. Biol. *71*, 83 (1922).
[4] S. RANZI and L. JANESELLI, Rend. R. Ist. Lombardo Sc. e Lett. (Cl. Sc.) *74*, 403 (1941).

monsters in Mollusca[1]. NaSCN induces an increase of the ectoderm at the expenses of entoderm and mesenchyme in the sea urchin (animalization)[2]; increase of notochord in Amphibians[3] and Cyclostomes[4] and monsters with a large stomodaeum in Cephalopods[5]. Schematically we might state that SCN induces a hyperdevelopment of some embryonic presumptive parts with a reduction of other parts. Li on the other hand affects these same parts, inducing hypodevelopment in the former and hyperdevelopment in the latter.

Besides LiCl, other substances are able to induce vegetalization in sea urchins[6] and malformations of the cyclopic series in Amphibians[7]. Swelling ions, vital dyes and paranitrophenol induce animalization in sea urchins[8] and enlargement of the notochord in Amphibians[9]. The effect follows HOFMEISTER's series, for the Na salts SO_4> tartrate >Cl>I>SCN (Na_2SO_4 is most active in inducing cyclopic malformations; NaSCN is most effective in inducing enlargement of the notochord). Among the chlorides Li>Na>Mg (LiCl is most active in inducing cyclopia). The ability of these salt solutions to induce cyclopia (or vegetalization) parallels their ability to precipitate colloidal solutions. Accordingly, the more active is a solution in inducing a large notochord (or animalization), the more it is effective in dispersing colloidal solutions[10]. This conclusion stresses the idea that changes in embryonic determination are induced by a direct action of the ions on the proteins of embryonic protoplasm.

Total extracts in 1 m KCl of embryos or eggs of the frog and fractions containing euglobulin *a*, euglobulin *b* or pseudoglobulin show a decrease in viscosity when treated with NaSCN at the concentration inducing hyperdevelopment of the notochord. The same effect is induced by NaI and pyocyanine, which are also able to induce hyperdeveloped notochord. On the contrary LiCl and all the salt solutions which are able to produce monsters of the cyclopic series induce an increase in viscosity of these extracts. Extracts containing globular shaped particles (euglobulin *c*), show an increase in viscosity when treated with NaSCN, NaI or substances inducing cyclopia[1].

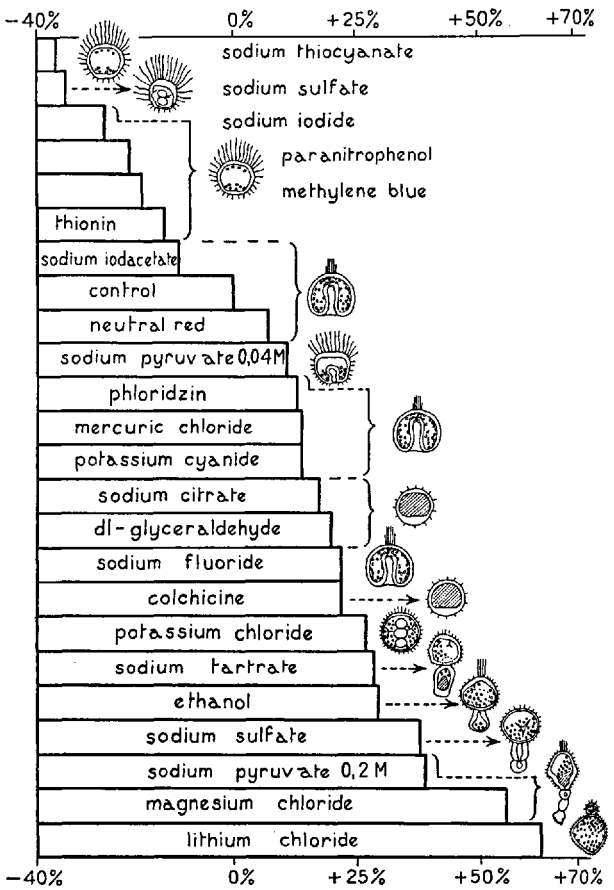

Fig. 4. – Scheme of the action of some substances on the viscosity of euglobulin *a* + *b* solutions of sea urchin eggs. The specific viscosity in presence of the mentioned substances is represented by rectangles. The larvae, obtained by the action of these substances, are represented at the right side. The base of the rectangle is the specific viscosity; that is conventionally indicated as zero for the control. Bases show the change in per cent of the viscosity corresponding to each substance. Good animalization is obtained for a decrease in viscosity above 14 per cent and good vegetalization for an increase in viscosity above 28 per cent (from AROSIO, CITTERIO, RANZI, and TOSI, 1949).

The same holds true for the proteins extracted from sea urchin eggs (fig. 4)[2]. The substances inducing animalization are able to induce a decrease in viscosity of the solutions containing fibrillar shaped proteins. The substances inducing vegetalization are able to induce an increase in viscosity of the same proteins. NaSCN, NaI, methylene blue, thionin, paranitrophenol, and under some circumstances Na_2SO_4 are animalizing agents. On the contrary LiCl, $MgCl_2$, Na-tartrate (neutral), ethanol and, under some circumstances, Na_2SO_4 are vegetalizing agents. KCl colchicine, Na-citrate, *d,l*-glyceraldehyde induce a slight vegetaliza-

[1] S. RANZI, Pubb. Staz. Zool. Napoli *9*, 81 (1928). – C. P. RAVEN, Biol. Rev. *23*, 333 (1948).

[2] P. E. LINDAHL, Acta Zool. *17*, 179 (1936).

[3] The statistic figures of hyperdevelopment of notochord in *Rana esculenta L.* (CORTI unpublished) expressed as number of nuclei at tail bud stage are:

	50 embryos untreated: controls	50 embryos treated with 0·05 m NaSCN
Average number of nuclei in the notochord	1107	1233
σ	± 172	± 202
Difference in the means	126	
σ_d	$\pm\sqrt{\frac{172^2}{50}+\frac{202^2}{50}}=\pm 37$	
Per cent increase of the number of nuclei induced by NaSCN	11·4	

[4] S. RANZI, Nature *155*, 578 (1945).

[5] S. RANZI, Boll. Soc. ital. Biol. sper. *19*, 68 (1944).

[6] E. TAMINI, Rend. R. Ist. Lombardo Sc. e Lett. (Cl. Sc.) *76*, 363 (1943).

[7] E. TAMINI, Roux'Arch. *142*, 455 (1943).

[8] E. TAMINI, Monit. Zool. ital. *52*, 81 (1941).

[9] S. RANZI, Naturwiss. *30*, 329 (1942).

[10] S. RANZI, Boll. Soc. ital. Biol. sper. *18*, 218, 314 (1943).

[1] R. AROSIO, P. CITTERIO, P. MENOTTI, S. RANZI, and F. SEMENZA, Riv. Biol. *38*, 153 (1946). – P. CITTERIO and S. RANZI, Rend. Acc. Naz. Lincei (Sc. Fis.) (8) *7*, 254 (1949).

[2] R. AROSIO, P CITTERIO, S. RANZI, and L. TOSI, Rend. Ist. Lombardo Sc. e Lett. (Cl. Sc.) *82*, 143 (1949).

tion. $HgCl_2$, Na-iodacetate, phloridzin, NaF, KCN have neither animalizing nor vegetalizing effect. Now, NaSCN, NaI, methylene blue, paranitrophene and thionin, at the concentrations active in animalizing, induce a decrease in viscosity of the solutions of sea urchin eggs' euglobulin $a + b$. LiCl, $MgCl_2$, ethanol and tartrate, at the concentrations active in vegetalizing, induce an increase in viscosity of the solutions of sea urchin eggs' euglobulin $a + b$. On the other hand KCl, colchicine, citrate, dl-glyceraldehyde, at concentrations inducing a vegetalization of low degree induce an increase in viscosity smaller than that induced by LiCl, $MgCl_2$, ethanol, tartrate. $HgCl_2$, iodacetate, phloridzin, KCN, at the concentrations permitting development, induce very small changes in viscosity of the solutions of sea urchin eggs' euglobulin $a + b$. NaF, at the highest concentration permitting normal development, induces an appreciable increase in viscosity of solutions of euglobulin $a + b$; evidently, the permeability conditions of the ion F, which precipitates in presence of Ca, induce the entrance only of a small amount of F into the cells. In the in vitro experiments, the F of the solution acts on the proteins extracted in KCl and after precipitation dissolved in KCl; whereas in the living embryo only a small amount of the F of the solution can act on the cellular proteins. Therefore the two kinds of results do not seem to be comparable. High increase in viscosity is induced by 0·3 m Na_2SO_4; but 0·03 m Na_2SO_4 induces a remarkable decrease in viscosity of the same solutions of euglobulin $a + b$ (fig. 5). Both concentrations are compatible with development, and it is probable that they correspond to the observed animalization and vegetalization.

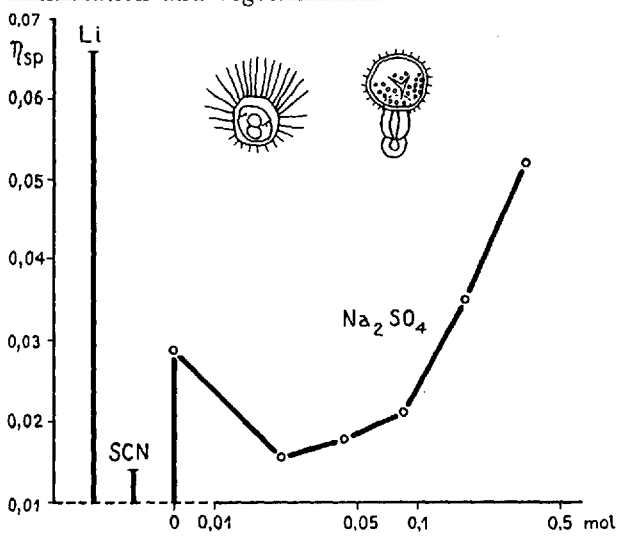

Fig. 5. – Specific viscosity (on the ordinate) of a solution of euglobulin $a+b$ from sea urchin egg for different concentrations of Na_2SO_4 in the solvent (on the abscissa). The two vertical segments on the left side show the viscosity of the solution with Li and SCN. Two larvae one animalized and the other vegetalized by the action of Na_2SO_4, are represented (from AROSIO, CITTERIO, RANZI, and TOSI, 1949).

HÖRSTADIUS[1] was able to induce both animalization and vegetalization of sea urchin embryos with some amino acids. In some experiments carried out with the same method used for analysis of the effect of salts, lysine, an animalizing amino acid, induced a decrease in viscosity of solutions of euglobulin $a + b$. Instead valine, leucine and glutammic acid, vegetalizing substances, induced an increase in viscosity. The results of these experiments seem to point out that amino acids do not act as amino acids as such on the proteic metabolism, which is operative during embryonic determination, but affect proteins through the same mechanism as SCN and Li.

All these reported data show that animalization of sea urchins and hyperdevelopment of the notochord of the Amphibians are produced by substances inducing a decrease in viscosity of fibrillar folded (or fibrillar) proteins extracted from embryos. On the contrary vegetalization of sea urchins and reduction of notochord of the Amphibians are produced by substances inducing an increase in viscosity. A parallel is demonstrated between effect of substances on embryonic proteins and effect on determination. The embryonic proteins, reacting specifically with substances inducing a change in determination, are fibrillar folded (or fibrillar) proteins. The ability of these proteins to react to all the above mentioned substances is due to the shape of their particles. Fibrillar protein extracted from the adult organism reacted in the same way[1]. Consequently the ability of the embryonic fibrillar proteins to react to the tested substances is due to the shape of their particles and not to any other property[2]. The conclusion is that fibrillar shaped proteins preside over the embryonic determination.

Let us now take into consideration the other mechanisms acting in embryonic determination. Sodium pyruvate, at low concentrations, animalizes sea urchin embryos and induces a slight increase in viscosity of the embryonic extracts. Pyruvate appears in the chain of carbohydrate breakdown; its animalizing effect can be interpreted as an influence of carbohydrate metabolism in animal development, in accordance with the idea of the Stockholm school[3]. It seems, however, that changes of the proteins precede the animal metabolic differentiation[4].

The data now referred to, show that the decrease in viscosity induced by SCN and I is related to an irreversible depolymerization of the fibrillar proteins. Therefore it is reasonable to conclude that a depolymerization of fibrillar proteins takes place during determination of the ectoderm of sea urchins and during determination of the notochord of the Amphibians. Such a

[1] S. HÖRSTADIUS, Pubb. Staz. Zool. Napoli *21*, (suppl.) 131 (1949).

[1] P. CITTERIO and S. RANZI, Rend. Acc. Naz. Lincei (Cl. Sc.) (8) *3*, 150 (1947).

[2] S. RANZI, Exp. Cell Research, Suppl. *1*, 555 (1949).

[3] P. E. LINDAHL, Quart. Rev. Biol. *17*, 213 (1942).

[4] R. AROSIO, P. CITTERIO, S. RANZI and L. TOSI, Rend. Ist. Lombardo Sc. e Lett. (Cl. Sc.) *82*, 143 (1949).

conclusion is in agreement with the results of YAMADA[1], who, treating ventral blastoporal lip with a higher p_H, was able to induce notochord formation; the high p_H depolymerizes the embryonic $a + b$ fraction of euglobulin, as will be discussed later.

The electron microscope pictures of actomyosin treated with Li[2], show piling up threads. It is not impossible that such a transformation inhibits the depolymerization and stops the formation of the ectoderm in sea urchin and the formation of the notochord in Amphibians.

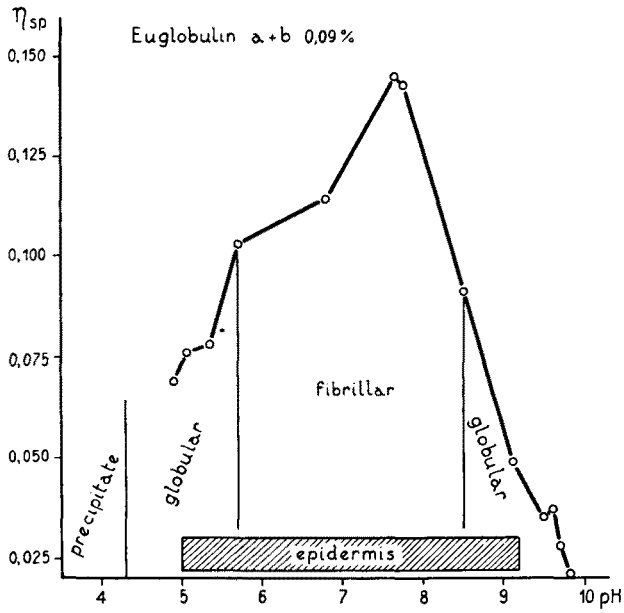

Fig. 6. – Viscosity of a solution of euglobulin $a + b$ from frog embryos at several p_H values. At the different p_H values, the shape of protein particles is indicated as it appears by the viscosimetric method. The shaded rectangle corresponds to the p_H values permitting the development of the ectoderm in epidermis according to HOLTFRETER. At lower and higher p_H values neural formations are evocated (from CITTERIO and RANZI, 1949).

The interpretation of the transformations induced by Li and SCN is in accordance with the changes in viscosity induced by Li and SCN in eggs of sea urchins. Unfertilized eggs of sea urchins, when centrifuged after being submitted to treatment with LiCl, show less tendency to stratify than the untreated eggs. On the contrary eggs treated with NaSCN show higher tendency to stratify[3].

[1] T. YAMADA, Biol. Bull. *98*, 98 (1950).
[2] S. RANZI, Nature *160*, 712 (1947).
[3] S. ABRUZZESE SGARLATA, Ric. Sc. e Ric. *17*, 473 (1947).

Finally, as to the evocation of the nervous system in Amphibia, it is well known that nervous system is induced by underlying structures. HOLTFRETER[1] has shown that nervous differentiation can be obtained from presumptive ectoderm of *Triturus* by treating it with a solution of p_H values lower than 5·0 or higher than 9·2. Between these two values of p_H, presumptive epidermis develops into epidermis. At p_H values lower than 5·7, or higher than 8·5, the fibrillar folded euglobulin $a + b$ is depolymerized into globular particles (fig. 6). It seems that the nervous differentiation in the experiments of HOLTFRETER corresponds to a depolymerization of fibrillar shaped proteins. In this depolymerization, the reaction of the presumptive neural cells to the evocating influence can be seen[2].

Riassunto

Trattando una soluzione di una proteina globulare con un leggero agente denaturante (p. e. 0,05 mol. KSCN nella soluzione totale), si osserva un aumento di viscosità rispetto al campione controllo. Se si fa la stessa cosa con una soluzione di una proteina a particelle filamentose si osserva una diminuzione di viscosità (fig. 1). Questo metodo è più sensibile della birifrangenza di flusso perchè può essere usato a concentrazioni più diluite. Dalla comparazione tra il metodo viscosimetrico e la birifrangenza di flusso si possono distinguere quattro forme di particelle proteiche (vedi tabella). 1° Particelle globulari (es. emoglobina); 2° Particelle che in certe condizioni appaiono globulari in altre filamentose (es. euglobulina b dell'embrione); 3° Particelle filamentose (es. actomiosina); 4° Particelle a graticciata (es. timonucleoistone rigido). Le ultime tre sorta di particelle possono venir rotte in più piccole unità che coi nostri metodi appaiono globulari; queste unità sono tenute insieme da legami rotti per azione di ioni imbibenti e pertanto non da legami peptidici.

Durante lo sviluppo embrionale alcuni processi sono accompagnati da trasformazione di proteine filamentose. Così la determinazione dell'ectoderma dei ricci di mare, quella della corda di Anfibi e Ciclostomi, l'induzione del tubo neurale degli Anfibi appaiono accompagnati da depolimerizzazione di proteine filamentose, mentre la determinazione dell'entoderma dei ricci di mare sembra accompagnata da un ammassamento di queste proteine. Questi dati ci portano alla conclusione che proteine, che possono presentarsi in forma filamentosa, hanno un ruolo di primo piano nella determinazione embrionale.

Sono anche state studiate le trasformazioni delle diverse frazioni proteiche nel corso dell'accrescimento della rana e del pollo.

[1] J. HOLTFRETER, J. exp. Zool. *106*, 197 (1947).
[2] P. CITTERIO and S. RANZI, Rend. Acc. Naz. Lincei (Sc. fis.) (8) *7*, 254 (1949).