THE EXISTENCE OF GLOBULIN X AS AN INDIVIDUAL PROTEIN

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In 1933 Weber [9, 10] suggested the name globulin X for that part of the protein of expressed muscle juice which is precipitated after prolonged dialysis of the juice against distilled water. The same protein, according to Weber's findings, is present also in saline extracts of striped muscles of both high and low ionic strength.

If pulped muscle is extracted with 0.6M KCl at pH = 8.2, and the extract is then dialyzed against a highly diluted phosphate buffer, when the ionic strength of the solution reaches a sufficiently low value, myosin (actomyosin) is the first to be precipitated. With a 0.04M concentration of CKl and at pH = 7.0, myosin (acetomycin) may be completely separated from the other muscle proteins. With a further fall in the concentration of KCl (below 0.005M) globulin X begins to be precipitated. Proteins of the myogen group and myoalbumin remain in solution. Globulin X belongs, therefore, to the group of water-insoluble proteins and, in practice, is a usual component of the muscle protein fraction extracted from pulped muscle by solutions of low ionic strength.

The total content of globulin X obtained by this method, according to Weber's findings, is about 20% of all muscle proteins. In expressed muscle juice, in the nitrogen of the globulin X is contained about 12-13% of the total protein nitrogen. The globulin X precipitated during dialysis may be redissolved only partially, probably because of the partial denaturation of the protein at the time of dialysis.

In spite of the great length of time elapsing since the description of globulin X by Weber, little is yet known about the nature and properties of this protein. According to the findings of Weber and Meyer [9, 10], globulin X possesses normal low viscosity, does not show double diffraction nor form threads when a solution of it is forced through capillary tubes into distilled water. Its isoelectric point is about 5, and its molecular weight, according to Deuticke [5], is 140,000-180,000.

On electrophoregrams globulin X is a component of the myogen fraction. On electrophoresis of muscle extracts with low ionic strength, no special component was found which corresponded in its properties to this protein [3]. Connel [4], by means of electrophoresis, subdivided the globulin X fraction of the muscles of the trout into three components.

In the present paper we describe the results showing the quantitative content of globulin X in muscle juice obtained by expression of the striped muscles of embryos, newborn and adult rabbits, the percentage content of globulin X in the different fractions of muscle juice, salted out by means of ammonium sulfate, and also the results of electrophoretic examination of the water-insoluble muscle globulins.

EXPERIMENTAL METHOD

In order to obtain muscle juice we used the striped muscles of the rabbit (spinal muscles and muscles of the limbs). Separate experiments were performed on the muscles of the heart and stomach. The excised muscles were freed from visible fat and connective tissue septa, washed to remove blood with cold physiological saline and pulped in a mincing machine.

The pulped muscle was immersed in an ice-salt mixture at a temperature of 10-12° until it was frozen to a firm consistency. The frozen pulp was cut into small pieces with a scalpel — "shavings" — ground in a cold mortar into a snow-like mass, and then expressed in a metal mold, also previously cooled.

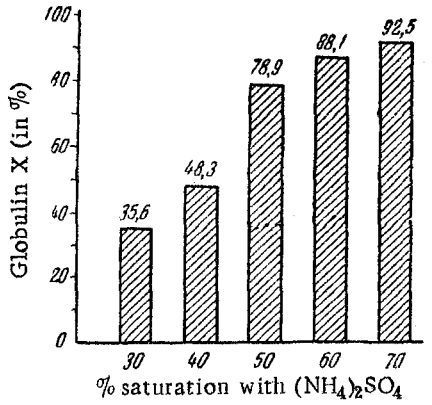

Fig. 1. Amount of globulin X in the precipitate after salting out the proteins of muscle juice with ammonium sulfate (in % of the total globulin X content of muscle juice).

Expression of the muscle juice was conducted at first under low pressure, which was then gradually increased to 50-60 atmospheres. The muscle juice thus obtained was dialyzed in a collodion bag against distilled water for 48 hours at a temperature around 0°. The precipitate — globulin X — was separated by centrifugation and washed three times with cold distilled water. In order to obtain a solution of globulin X the precipitate was treated with a buffer solution with pH = 5.8 and an ionic strength of 0.3.

To shed light on the question at what concentrations of $(NH_4)_2SO_4$ salting out of globulin X took place, saturated ammonium sulfate solution was added to 5 samples of muscle juice (each of 6 ml) in volumes so that the final concentration of $(NH_4)_2SO_4$ in the test samples was 30, 40, 50, 60 and 70% saturation respectively. The salt solution was added to the muscle juice gradually, with slow agitation. Salting out took place in wide test tubes for 12 hours at a temperature from 1° to 4°.

The protein precipitate was separated by filtration through filter paper and was then completely redissolved in a phosphate buffer with pH = 5.8 and ionic strength 0.3.

The globulin X content was determined in the filtrates and precipitates thus obtained, for which purpose a known volume of filtrate and dissolved precipitate was dialyzed for 48 hours against distilled water at a temperature of around 0°. The amount of globulin X precipitated was determined by a gravimetric method (after drying to constant weight at a temperature of +105°) or by the micro-Kjeldahl method after redissolving the protein precipitate.

By means of paper electrophoresis the fractional composition of the proteins of the crude muscle juice, of the muscle juice after removal of the globulin X from it by dialysis, and of the globulin X obtained by dissolving the precipitate formed by dialysis of the muscle juice against distilled water in a buffer solution with pH = 5.8 and an ionic strength of 0.3, was investigated.

Electrophoresis was carried out for 18 hours at a current strength of 0.8 ma per cm cross section of the paper strip and at a voltage of 6-8 v/cm.

EXPERIMENTAL RESULTS

According to our findings, the muscle juice of the striped muscle of the adult rabbit contained 9-10% of protein; the globulin X fraction accounted for about 13% of the total protein content of the muscle juice.

After being precipitated during dialysis, globulin X lost its solubility to a large extent. For instance at pH = 5.8 and an ionic strength of 0.3, only about 40% of the precipitate of globulin X was dissolved, and the remainder lost its solubility under these conditions. This fact agreed with the results of Weber's research.

The globulin X content of muscle juice, according to our findings, depended on the age of the animal and on the type of muscle. The muscle juice of cardiac muscle contained a larger amount of globulin X than the striped muscle (about 20%). In the juice expressed from the muscle of the stomach the globulin X content showed wide variations (from 15 to 30-35%). Characteristic changes in the globulin X content were observed in the muscle juice of the striped muscles in ontogenesis (see table).

In Fig. 1 are shown the results of the experiment in which globulin X was salted out from the muscle juice of the striped muscles of the adult rabbit at different degrees of saturation with ammonium sulfate, from which it may be seen that the smallest quantity of globulin X (35.6% of its total content in the muscle juice) was salted out at 30% saturation with ammonium sulfate. At higher degrees of saturation the amount of globulin X

salted out gradually increased. At 70% saturation 92.5% of the globulin X was precipitated, and even at this degree of saturation it was not fully salted out. The largest amount of protein, globulin in character, was precipitated in the zone of 40-50% saturation with ammonium sulfate (Fig. 1).

Globulin X Content of the Muscle Juice of the Striped Muscle of the Rabbit (as a percentage of the total protein content of the juice examined)

Embryo (25 days)	Newborn	Adult
42	35	13

Globulin X was salted out from the muscle juice at all concentrations of $(NH_4)SO_4$ as a mixture with other proteins. The absolute amount of slated out globulin X rose with an increase in the concentration of ammonium sulfate, whereas the relative content decreased under these circumstances. The highest percentage content of globulin X in the precipitate was at 30% saturation. There was thus no doubt that the globulin X did not have a definite enough zone of salting out.

In Fig. 2 are shown the electrophoregrams of the proteins and the corresponding graphs of crude muscle juice, muscle juice after removal of the globulin X by dialysis and the globulin X preparation.

Several proteins are known to enter into the composition of muscle juice. On the electrophoregram (Fig. 2, a) are seen four main bands, one of which is faintly marked and situated at the starting line, and evidently corresponds to the portion of the most readily denatured protein fraction. Since the ionic strength of the muscle juice did not exceed 0.2, only "water-soluble"* muscle proteins could be present in the expressed juice.

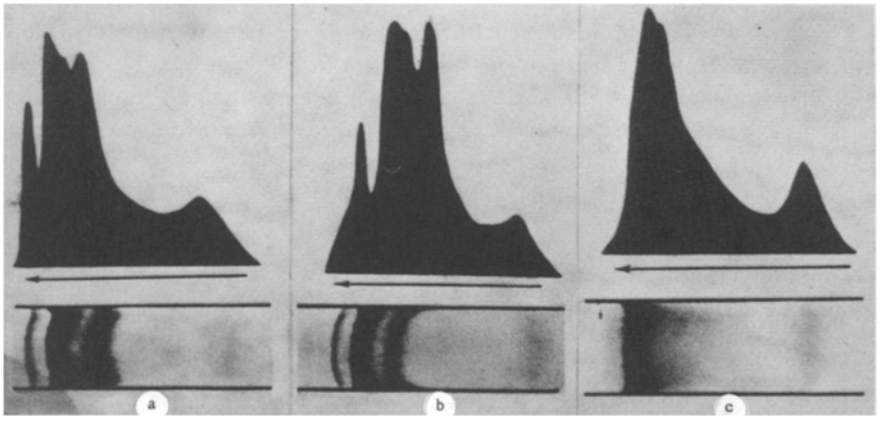

Fig. 2. Electrophoregrams: a) of the proteins of muscle juice of the striped muscles of the adult rabbit; b) of the proteins of muscle juice of the striped muscles of the adult rabbit after removal of the globulin X; c) of the globulin X isolated from the muscle juice of the striped muscles of the adult rabbit.

According to the findings of I. I. Ivanov and his co-workers [1], muscle juice contains neither myosin nor actomyosin. There are also reports [2, 6, 7, 8] that in muscle extracts with an ionic strength of 0.15, during free electrophoresis, three groups of "water-soluble" proteins are revealed, each of which consists of several components (m, n, l, first group; K_1 and K_2, second group; J, Sp, h, third group).

At the present time it is not yet possible to assert definitely to which components of the proteins, soluble in saline media with a low ionic strength, obtained by means of free electrophoresis, correspond all the stains which appear during paper electrophoresis of muscle juice.

* By the term "water-soluble" proteins we designate proteins soluble in saline media with a low ionic strength.

In all probability, judging by the quantitative content and by the order of arrangement on the strip of paper, the three fractions of muscle juice of the adult rabbit (Fig. 2, a) correspond to the components h and m, n and l, and possibly k. Myoalbumin (h) may be found in a significant amount only on the electrophoregrams of the muscle juice proteins of embryonic and newborn rabbits (Fig. 3).

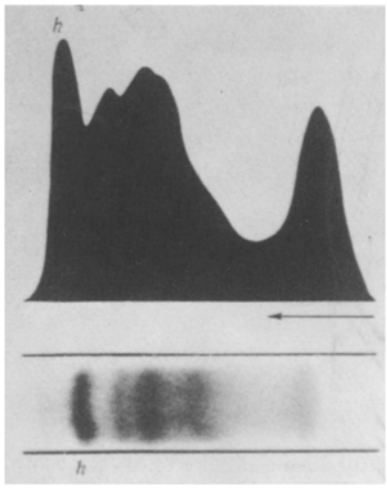

Fig. 3. Electrophoregram of the proteins of the muscle juice of the striped muscles of a newborn rabbit.

After removal of the globulin X from the muscle juice, not one of the fractions disappeared from the electrophoregram (Fig. 2, b). The graphs of the two electrophoregrams are hardly distinguishable from each other; the peak corresponding to the "starting" fraction was somewhat lowered after removal of the globulin X.

Globulin X obtained by redissolving the precipitate from dialysis of muscle juice against distilled water in a buffer solution with pH = 5.8, was found to consist of two main fractions – the larger of them evidently nonhomogeneous, which is shown clearly on the graph (Fig. 2, c). It must also be emphasized that globulin X may possibly have a still more complex fractional composition, since the electrophoretic investigation was carried out only with the soluble part of this protein.

It thus follows from our findings that globulin X does not exist as an individual protein.

The protein described by Weber under the name globulin X is in fact a mixture of globulins with different physiological properties. These proteins do not have a sufficiently definite zone of salting out (in the presence of ammonium sulfate) and their electrophoretic mobilities, although close, are nevertheless not identical.

A certain number of proteins which differ from the true globulins by their solubility in a half-saturated solution of ammonium sulfate enter into the composition of Weber's globulin X.

SUMMARY

The quantitative content of globulin X was investigated in the muscle juice obtained by pressing the striped muscles of embryos, newborn and adult rabbits. The globulin X percentage was also determined in various fractions of the muscle juice salted out with ammonium sulfate. Electrophoretic investigation of water-insoluble muscle globulins was also conducted. The data obtained led the authors to a conclusion that globulin is not an individual protein but a mixture of different globulins varying according to their physicochemical properties. Electrophoretically, globulin is not detected as a separate component. Its content in the striped muscle juice changes during the process of ontogenesis.

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