CHANGES IN THE SORPTIVE PROPERTIES OF CERTAIN TISSUES OF THE RAT DURING AGING

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(Received March 3, 1958. Presented by Active Member Acad. Med. Sci. USSR, V. N. Chernigovskii)

According to the research carried out at Khar'kov University with aging a gradual extinction of the processes of self-renewal takes place [6, 7]. Since protein is the main component of the protoplasm and the material basis of all vital processes, it is natural to connect these changes in metabolism with qualitative and quantitative changes taking place in the protein substrate.

Ruzicka [15-17] considered that during aging there is primarily a physicochemical alteration in the protein: there is a fall in its electric charge and in its degree of ionization, in consequence of which there develops dehydration, thickening of colloids, replacement of more labile by stable compounds, increased viscosity and a reduction in the degree of dispersion. This complex group of changes as a whole was called by Ruzicka protoplasmic hysteresis, and on it was based his colloid-chemical theory of aging.

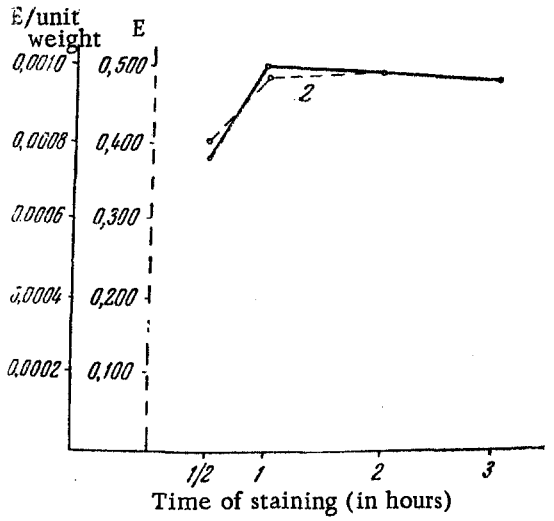

Fig. 1. Sorption of phenol red by muscle (1) and corneal (2) tissue of rats after staining for different lengths of time. E – extinction

Recently, in work by I. N. Bulankin and M. A. Blyumina [3], the hypothesis has been put forward of an "age increase in the cyclic structures in the protein molecule or an age stabilization of the cyclic structures already present in the protein molecule."

We thought it interesting to consider the problem of the qualitative changes occurring in proteins during the process of aging, on the basis of the denaturation theory of excitation and injury, put forward by D. N. Nasonov and V. Ya. Aleksandrov [8].

It was shown by D. N. Nasonov and his co-workers, in a series of investigations, that under the influence of various stimuli – i.e. in response to excitation and injury – the protoplasm begins to adsorb vital stains more intensively [5, 8, 9, 11, 12].

The increased sorption of acid and basic stains is associated with denaturation changes in the protein, and results from the appearance of new, ionized groups in the protein molecules. Possibly, during aging, the tissues cells reach a state which, in its nature, closely resembles that which arises in response to the action of stimuli, so that similar changes (of the denaturation type) must take place then in the protoplasm, and in particular an increased sorption of stains would be expected.

The present work was devoted to an experimental verification of this hypothesis. We also thought that it would be interesting to find out whether the blood proteins are also affected in a similar manner.

EXPERIMENTAL METHOD

The investigation was carried out on white rats which had been reared in the laboratory. Tissues from rats of four ages were investigated: 3, 6, 18 and 24 months.

The experimental material consisted of the muscles (soleus and extensor digitorum longus), the cerebral hemispheres, the cornea and the blood serum. The animals were killed by decapitation. Blood was collected in tubes, in which the serum was allowed to separate.

TABLE 1

Sorption of Vital Stains by Muscle Tissue of Rats of Different Ages (in Extinctions per mg Dry Tissue) (the arithmetic mean ± mean square deviation is given)

Object and dye	Age, in months			
	3	6	18	24
Soleus muscle (phenol red, 0.06%)	0.0040 ± 0.00014	0.0052 ± 0.00011	0.0062 ± 0.00037	0.0081 ± 0.00032
Number of experiments	14	24	13	8
Extensor digitorum longus muscle (neutral red, 0.05%)	0.0033 ± 0.00012	0.0042 ± 0.00017	0.0066 ± 0.00034	0.0054 ± 0.00025
Number of experiments	11	15	9	11

TABLE 2

Sorption of Pheno Red (0.05%) by Brain and Corneal Tissues of Rats of Different Ages (in extinctions)

Object	Age, in months			
	3	6	18	24
Brain tissue	0.234 ± 0.013	0.318 ± 0.015	0.368 ± 0.016	0.460 ± 0.025
Number of experiments	13	15	11	10
Corneal tissue	0.306 ± 0.009	0.315 ± 0.008	0.353 ± 0.021	0.411 ± 0.016
Number of experiments	14	16	10	11

TABLE 3

Sorption of Methylene Blue (9 mg%) by the Serum Proteins of Rats of Different Ages (quantity of dye in mg/100 mg of albumin)

Object	Age, in months			
	3	6	18	24
Blood serum	273.7 ±18.2	410.7 ±26.8	511.5 ±38.9	533.1 ±12.1
Number of experiments	10	11	8	8

The organs for testing were placed in Ringer's solution (muscles and brain for 5 minutes, cornea for 10 minutes).

In the investigation of the sorptive properties of the muscles, brain and cornea a quantitative method of vital staining was used. The soleus muscle, brain and cornea were stained with an acid stain – phenol red (the concentration used for the brain and cornea was 0.05%, and for the muscle 0.06%), and the extensor digitorum longus muscle was stained with a basic stain – neutral red (0.05%). In staining all the tissues care was taken as far as possible to avoid injury.

For staining the muscles the solutions were aerated; for staining the brain the lay of stain above it did not exceed 2 mm in thickness. After extraction of the tissues from the stain, no perceptible abnormalities were found under the microscope.

The criterion of a good condition of the muscle which was used was only little changed in its excitation throughout the experiment. Its fall did not exceed 20%.

The soleus muscle was stained in an incubator at 35° for one hour, i.e. until the establishment of equilibrium in the distribution of the acid stain between the muscle and the solution (Fig. 1). The extensor digitorum longus muscle was stained in the same way for 5 minutes.

After they had been removed from the stain the muscles were rinsed, dried on filter paper and extracted with 70% alcohol acidified with 7% sulfuric acid in the case of the acid stain and acidified with 2% sulfuric acid in the case of the basic stain. In both cases the extracted dyes were estimated photometrically on the photoelectric colorimeter, after which the muscles were dried to constant weight and the sorption of the stain was calculated in relative units (extinction per mg dry tissue).

The cornea was stained in toto for 40 minutes at 37° (see Fig. 1), after which the tissue was washed, and pieces were cut from it with a circular punch (d = 4.5 mm), which were placed in acidified alcohol in order to extract the stain.

The brain was stained in the same way, washed in Ringer's solution, and pieces were cut from the parietal and temporal areas of the cerebral hemispheres, by means of a circular punch (d = 8 mm), and used for extraction of the stain.

The extracts from the brain and cornea were estimated photometrically in small dishes with a Pulfrich's photometer. The quantities of stain adsorbed were compared as "extinctions" shown by the readings of the photometer.

For the investigation of the sorptive properties of the serum proteins, the method used was that of diffusion of free dye in a gelatin gel, as devised by A. D. Braun [2] and slightly modified by ourselves.

The serum protein concentration was determined by the biuret method, and the albumin-globulin ratio nephelometrically [1]. The serum was stained by a basic dye – methylene blue – in an initial concentration of 9 mg%. Fixation of dye was calculated as mg/ 100 mg of albumin, since of the serum proteins, it is only the albumins which fix the dye [13].

The experiments were carried out over a period of 3 years, at different seasons. Control experiments were performed at the same time on 6-month old animals. The sorptive power of the tissues of the 6-month old rats was practically unchanged.

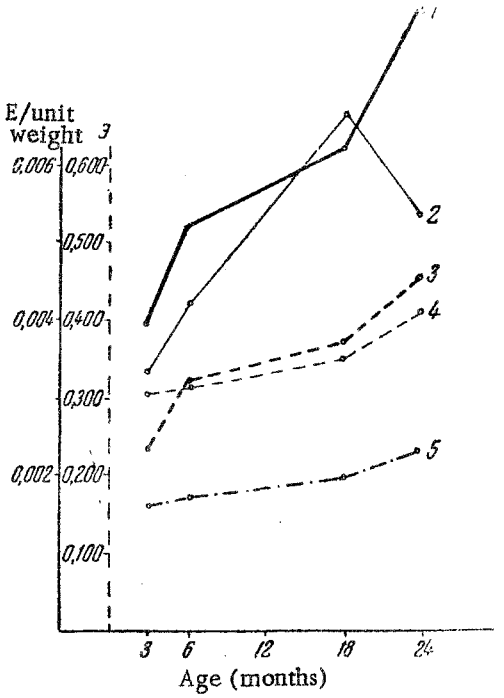

Fig. 2. Changes in the sorptive properties of muscle tissue (soleus, stained with phenol red – 1; extensor digitorum longus, stained with neutral red – 2), brain (3) and corneal (4) tissue of rats of different ages. Changes in the pigment content of the brain tissue during aging (5). E – extinction.

EXPERIMENTAL RESULTS

As can be seen from the data in Tables 1-3, an increase in the sorptive power of the tissue and serum proteins took place during aging. The sharpest increase in sorption of dye was observed in the tissues of the brain, muscles and cornea at the age of 18-24 months.

If the sorptive power of the tissue of the 3-month old animals was taken as 100%, then the sorption of dye by tissues of the 2-year old animals was increased in the brain by 96.6%, in the cornea by 34.3%, in the muscle for phenol red by 102.5% and for neutral red by 63.6%.

Attention must be drawn to a peculiarity in the change in sorption of basic dye, consisting of the fact that the sorptive power of the muscle tissue toward neutral red rose comparatively abruptly in the period from 3 to 18 months, and then fell considerably at 24 months.

The brain tissue stained extremely weakly, so that a considerable proportion of the extinction during photometry was due to pigment, especially in the younger animals. With increasing age the pigment content of the brain tissue rose (Fig. 2), although the curve of pigment content rose less abruptly than the curve of sorption of dye.

Table 3 illustrates the changes in the sorptive properties of the serum proteins during aging. The character of these changes was the same as that in the tissue proteins of the brain, cornea and muscles, for the sorptive power of the serum proteins of the older animals 2 years) was 94.8% greater than that of the younger animals (3 months)

(Fig. 3). The sharpest changes took place at the age of 3-6 months; the sorptive properties of the serum proteins of the animals aged $1\frac{1}{2}$ and 2 years were practically indistinguishable from each other.

The increase in sorption of dye by the living tissues of the rats with advancing age was evidence of definitive and substantial changes in the proteins on aging.

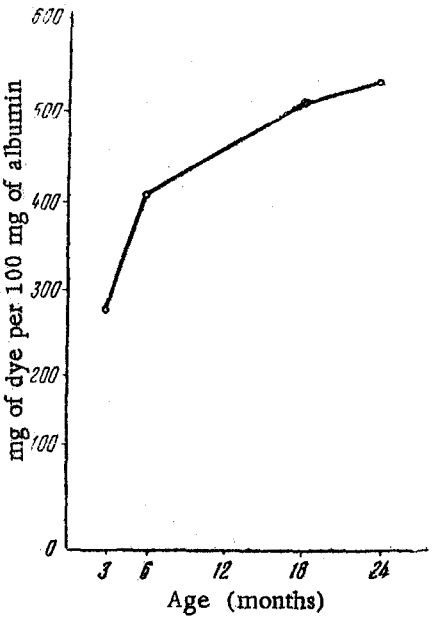

Fig. 3. Changes in the sorptive properties of the serum proteins of rats during aging.

There are several references in the literature to changes in the physicochemical and biochemical properties of proteins during aging, supporting the views of Ruzicka.

Bechhold [14], for instance, describes an increase in the viscosity of the protoplasm of cells during aging. Similar findings were reported by V. N. Nikitin, I. A. Babich and V. S. Lyubavina [10], during an investigation of the viscosity of the serum of cattle. According to the findings of A. V. Nagornyi [6], Hajek [18] and A. O. Voinar [4], the pH of the protoplasm moves in the direction of acid during aging.

If we compare the data in the literature with our experimental findings, it appears possible to postulate that during aging of the body denaturation of the tissue proteins takes place.

SUMMARY

The changes in the sorptive properties of the muscular, nervous, epithelial and connective tissues of white rats were studied in old animals. The quantitative method of vital staining was used for evaluation of the sorptive properties of these tissues. Both basic and acid stains were used.

It was established and statistically confirmed that the sorptive properties of the muscles, brain, cornea and proteins of the blood serum are increased with age, which points to the qualitative changes in the proteins of various tissues. A suggestion is made of the denaturating origin of these changes.

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