CHANGES IN THE METABOLITE CONTENT OF TISSUE PRESERVED BY V. P. FILATOV'S METHOD

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Tissue therapy is widely practised in hospitals in the Soviet Union and abroad. The practical application of the method of tissue therapy has to some degree determined its theoretical teratment. The mechanism of action of tissue transplants and the nature of the "biogenic stimulators" have not yet been satisfactorily explained. One way of shedding light on the chemical properties of the "materials of resistance" is by the study of the morphological and physiological changes in living tissues kept for various periods of time at a temperature of 2-4°C. Tissue preserved by Academician V. P. Filatov's method is, firstly, damaged tissue and secondly, is kept under conditions of partial asphyxia. It is known from reports in the literature that the nucleic acids, which play an important role in the vital processes of the body, are accumulated in tissue undergoing reversible damage [9, 10, 15, 17]. In conditions of asphyxia the glycogen content of the tissue diminishes and the lipid content increases [3, 6].

Our aim was to study the progress of the changes in the content of ribonucleic acid (RNA) in tissues during preservation. Furthermore, in several series of experiments a study was made of the changes in the glycogen and lipid contents of living tissues kept in conditions of hypothermia for various periods of time.

EXPERIMENTAL METHOD

The material used in the investigation was tissue from various organs (liver, tongue, intestine) from 28 guinea pigs and 8 rabbits, splenic tissue from cattle and human placenta. The material mainly used was liver from rabbits and guinea pigs. The tissues were preserved for various lengths of time (from one to ten days) by Academician V. P. Filatov's method, and after fixation they were examined for their content of RNA, glycogen and lipid.

In this investigation particular attention was paid to the study of the changes in the RNA content of the preserved tissue. According to data in the literature, one of the criteria of the RNA content is basophilia of the fixed tissue, which disappears during the action of the enzyme ribonuclease or of trichloracetic acid on the tissue [14]. Basophilia of the tissue, i.e. the ability of the fixed tissue to combine with a basic dye, was determined by Brachet's method in histological preparations and by photometry of alcoholic extracts of sections stained with a basic dye.

As several workers have shown [10, 11], changes in the combination of basic dye (diminution or increase of sorption of the dye) are found in association with changes in the content of basophilic substances (RNA) in the tissue. Arising from these investigations, in order to define the changes in the content of basophilic substances we also employed the method of photometry of alcoholic extracts of tissue sections stained with a basic dye. The results obtained by this method were compared with those obtained by Brachet's method.

In using the photometric method, we studied the basophilia of the tissue as follows: from tissue preserved for various periods of time at a low temperature, after fixation with 20% neutral formalin, sections were

prepared with a razor (in the case of the liver the area of the piece of tissue was 0.5 cm^2 and its weight from 45 to 55 mg). From 5-10 sections were taken from tissue for each day of preservation. Each section was stained with a 0.01% solution of neutral red, and decolorized with acidified alcohol. The amount of dye in the alcoholic extracts was judged by the extinction expressed in conventional units of a graduated photometer and calculated per 100 mg weight of tissue. Fixation of dye by unpreserved tissue, i.e. fixed immediately after decapitation of the animal, was conventionally taken as 100% (control). The changes in the fixation of dye by the tissue (decrease or increase) in the course of preservation were expressed as a percentage of the control.

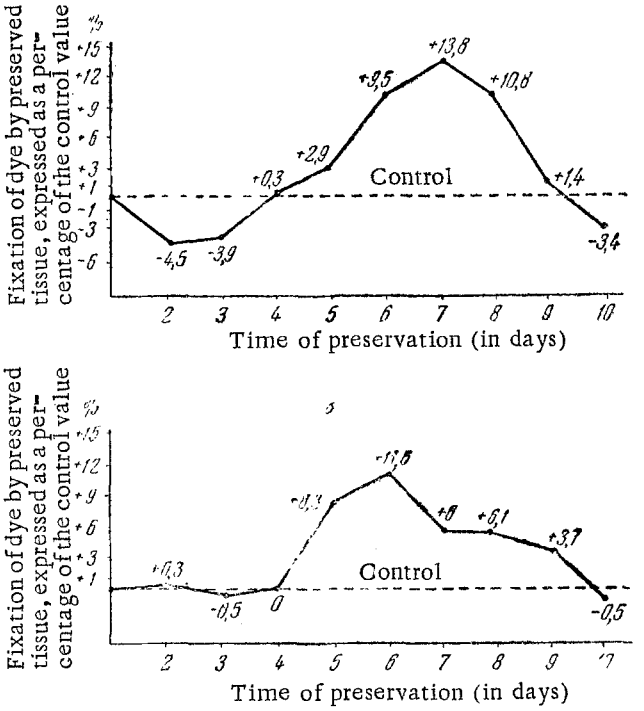

Fig. 1. Changes in the power of fixed tissue to combine with basic dye during the process of preservation of the tissue; a) liver tissue from guinea pigs (average of 23 experiments); b) liver tissue from rabbits (average of 8 experiments).

Glycogen was investigated in micropreparations obtained from sections of liver tissue from 5 rabbits by Best's method. Quantitative changes in the lipids were studied in preserved liver tissue from 5 rabbits and one guinea pig. The lipid was determined with Sudan III.

EXPERIMENTAL RESULTS

The experimental findings showed that in the process of preservation of liver tissue from rabbits and guinea pigs, the basophilia of the tissue as determined by the method of photometry undergoes the following changes (Fig. 1). The basophilia of the liver tissue, in the first 4 days of preservation, either remains the same as that of the unpreserved tissue, or even diminishes. Starting from the 5th day of preservation, the basophilia of the liver tissue of rabbits and guinea pigs increases and reaches a maximum for rabbit liver on the 6th day of preservation (11.6% ± 1.7 over the control value), and for guinea pigs liver – on the 7th day of preservation (13.8% ± 2.8 over the control value). After the 8th day the sorption of basic dye by the tissue begins to fall.

The same changes in the basophilia of the tissue in the process of preservation were clearly demonstrated by Brachet's method on micropreparations as by the method of photometry. In the first days of preservation, for instance (2nd-4th day) the basophilia of the liver tissue is no greater than that of control sections. On subsequent days (6th-8th day) a sharp increase in the intensity of staining of the sections of liver tissue is observed, after which the pyroninophilia begins to show a marked fall. Thus the determination of the basophilia of the tissue after various periods of preservation by the different methods gave compatible results.

The changes in the basophilia of the tissue in the course of its preservation are evidence of changes in its content of RNA. The progress of the changes in the RNA content of liver tissue of rabbits and guinea pigs, which we established, enabled us to subdivide the whole period of preservation into 3 periods. In the first period of preservation (from the 1st to the 4th day) the RNA content of the tissues shows no increase in comparison with that of unpreserved tissue, and the RNA content of liver tissue from guinea pigs even diminishes. In the second period of preservation (from the 5th to the 8th day) the RNA content of the tissues increases successively from day to day, reaching a well marked maximum on the 6th-7th day of preservation. Tissue in the second period of preservation, i.e. preserved for that length of time advised by V. P. Filatov for transplantation to patients, is characterized according to data in the literature [2, 4, 11, 16] by physiological processes which proceed with considerable energy. In the third period of preservation (after the 9th day) the RNA content of the tissue, according to our findings, falls sharply. It has been shown by work emanating from V. P. Filatov's school [2, 12] that profound autolytic processes develop in tissue which has been preserved for 9 days and more.

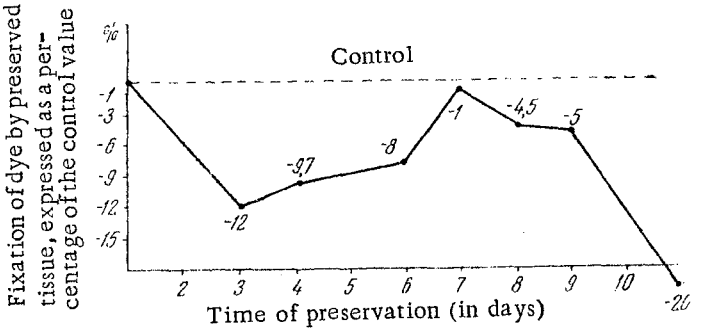

Fig. 2. Changes (in per cent) in the power of combination with basic dye (in the course of preservation) of fixed liver tissue from guinea pigs with avitaminosis in the process of preservation of the tissue. Mean results of 5 experiments.

In addition to studying the changes in the basophilia of the liver tissue in the course of preservation, we examined the changes in the basophilia of the tissue of other organs (spleen of cattle, human placenta, tongue and intestine from 7 guinea pigs and 6 rabbits). The basophilia of the tissue of these organs also increases during our second period of preservation (on the average from 10 to 33%). Growth of basophilic substances (RNA) in the preserved tissues may evidently take place by various methods. First of all its is admitted that there is an increase in the content of free RNA as a result of its liberation from its bond with protein. It is possible that an increase in the quantity of polynucleotides or of acid groups unconnected with protein may bring about intensification of the basophilia of the preserved tissue; it is also possible that rupture of the bond between neighboring nucleotides within the same molecule of nucleic acid may lead to an increase in the sorption of basic dyes by the tissue. The experimental results of S. M. Shibaeva [13] show that a process of depolymerization of nucleic acids takes place in preserved tissue. The fall in the basophilia of the tissue in the third period of preservation is due, in our opinion, to the departure of basophilic substances from the tissue. Flushing of RNA (basophilic substances) from tissue suffering damage has been observed by many workers [1, 7-9].

Our experimental findings from the study of the changes in the lipid and glycogen contents of preserved liver tissue from rabbits and guinea pigs showed clearly that from the first days of preservation the glycogen content of the tissue diminished sharply, whereas the lipid content rose. Our observation that there is a fall in the glycogen content and a rise in the lipid content confirms the findings obtained previously by V. P. Filatov's school [5].

We thus established that in tissue undergoing preservation quantitative changes take place in the contents of RNA, glycogen and fat. At strictly determinate periods of preservation there are regular changes in metabolism, evidently connected with the differing intensity of damage to the tissue. The changes in the metabolism on the 6th-7th-8th day of preservation are of the utmost interest, since it is tissue at this stage of preservation which is recommended by V. P. Filatov for transplantation. Our findings show that there is an increase in the RNA content of tissue at this period of preservation.

V. P. Filatov and his school consider that "materials of resistance" accumulate in preserved tissues taken from young and healthy animals; for this reason we studied the character of the changes in the RNA content of preserved liver tissue taken from healthy guinea pigs and from guinea pigs with avitaminosis. In full agreement with V. P. Filatov's views on the accumulation of biostimulators in preserved tissues taken from healthy animals, according to our findings during preservation of tissue an accumulation of RNA on the 5th-8th day takes place also, but only if the tissue is taken from healthy animals. The basophilia of liver tissue taken from 5 guinea pigs with avitaminosis did not exceed that of the control on any one day of preservation or in any single experiment (Fig. 2).

According to V. P. Filatov's concept of tissue therapy, the tissue of animals kept in arduous conditions of existence and without preservation, contains a large quantity of biostimulators. According to our findings, unpreserved liver tissue from 5 guinea pigs with avitaminosis contained significantly more RNA (on the average 20% more) than did the unpreserved liver tissue from healthy animals. Considering in the first place the important role of RNA in the vital processes, and in the second place the parallel changes in the therapeutic activity and the RNA content of preserved tissue, it may be postulated that RNA is a component part of the biostimulators. It is also admitted that the increase in the RNA content of preserved tissue may be used as a test of the preparedness of the tissue for transplantation to patients.

SUMMARY

The author studied the dynamics of the changes occuring in the content of basophilic substances (RNA) glycogen and fats in the tissues preserved by the method of academician V. P. Filatov. The total period of preservation may be subdivided into 3 stages. During the 1st stage of preservation (from the 1st to the 4th day) the quantity of the basophilic substances remains unchanged or decreased as compared to the control. The quantity of RNA is greatly increased from the 5th to the 8th day (the second stage), while after the 8th day the amount of RNA shows a rapid reduction. If the tissue is taken from sick animals basophilia does not increase during the process of preservation. The quantity of glycogen is sharply diminished from the very first days of preservation, while the quantity of fat rises.

By estimating the increased quantity of the basophilic substances in the preserved tissue one may judge the readiness of the tissue for transplantation to the patients.

LITERATURE CITED

[1] S. N. Aleksandrov, S. E. Manoilov and B. A. Orlov, Doklady Akad. Nauk SSSR, 83, 5, 725-728 (1952).

[2] M. A. Bazhenova, Collection of Scientific Papers of the Eye Clinic, 1, 2, 79-85, Odessa, 1936.*

[3] V. V. Barbarin, Biol. Zhur. 7, 2, 391-398 (1938).

[4] V. Ia. Batunina, Scientific Papers of the Gor'kii Institue of Dermatology and Venereology, 11, 25-28, Gor'kii, 1947.*

[5] L. E. Gelelovich, Scientific Material of the Ukrainian Institute of Experimental Ophthalmology, 64-65, Odessa, 1939.*

[6] L. N. Zhinkin, Transactions of the Petergof Scientific Institute, 6, 199-213 (1929).*

[7] L. F. Larionov and E. M. Brumberg, Doklady Akad. Nauk SSSR, 54, 3, 267-270 (1946).

[8] G. I. Roskin and V. Ia. Brodskii, Doklady Akad. Nauk SSSR, 89, 6, 1099-1102 (1953).

[9] A. N. Trifonova, Doklady Akad. Nauk SSSR, 61, 5, 917-920 (1948).

[10] A. N. Trifonova, Doklady Akad. Nauk SSSR, 85, 4, 941-944 (1952).

[11] A. N. Trifonova and B. M. Tikhomirov, Doklady Akad. Nauk SSSR, 85, 5, 1185-1188 (1952).

[12] V. P. Filatov, Tissue Therapy (Treatment by Biogenic Stimulators) Tashkent, 1948.*

*In Russian.

[13] S. M. Shibaeva, Morphological and Histochemical Changes in the Retina During Preservation. Candidate's dissertation, Moscow, 1954.*

[14] J. Brachet, Arch. Biol. 1940, v. 61. No. 3.

[15] J. Brachet, Symp. Soc. Exper. Biol. 1947, N. 1, pp. 207-224.

[16] Y. J. Chyr, J. Bronec and J. Kaninsky, Scripta med. 1953, 26. N 4-5, pp. 156-157.

[17] H. Hyden, Acta physiol. Scandinav. 1943, v. 6, supp. 17.

*In Russian.