THE ARGENTO- AND MERCURIMETRIC DETERMINATION OF THE CONTENT OF SH- GROUPS IN THE SERUM AND BRAIN HOMOGENATE OF THE CAT

Iu. M. Torchinskii

From the Laboratory of Histochemistry (Head – Prof. V. V. Portugalov) of the Institute of the Brain (Director – Active Member of the AMN SSSR S. A. Sarkisov) of the AMN SSSR, Moscow

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The amperometric method of titration of SH- groups with silver nitrate, introduced on 1946 by Kolthoff and Harris [7], has also been successfully applied to the analysis of several thiol compounds (cystein, glutathione and other) [8, 11], and to the determination of the content of SH- groups in different proteins [6, 10], in the blood serum [2, 12] and also in nonprotein blood filtrates and extracts of organs and tissues [3, 5]. K. V. Savich and V. A, Iakovlev [1] showed that it was possible to use this method in determining the content of SH- groups directly in a tissue homogenate. However Benesch, Lardy and Benesch [4] have recently expressed the opinion that titration of SH- groups of proteins with $AgNO_3$ in an ammoniacal buffer may give too low results because of "disappearance" of part (by oxidation) of the SH- groups by the action of the ammonium ion in an alkaline medium. Results confirming this point of view were obtained by the authors cited [4] and also by Ingram [6] during titration of the SH- groups of hemoglobin in a buffered solution containing 0.25 M NH_4OH and 0.05 M NH_4NO_3. In order to find out whether significant oxidation of the SH- groups of blood serum and brain homogenate does take place during titration with $AgNO_3$ in a mixed ammoniacal buffer, we carried out simultaneous titration of SH- groups with $HgCl_2$ in a phosphate buffer. Under these circumstances our findings differed from those of Kolthoff, Stricks and Morren [9], according to whom one mole of $HgCl_2$, during titration in a phosphate buffer in the presence of potassium chloride, combines with one mole of SH- groups in cystein, glutathione and serum albumin.

EXPERIMENTAL METHOD

Weighed samples of brain tissue (50-100 mg) were homogenized in a Potter glass homogenizer with 0.5 ml of distilled water, kept cold on ice; distilled water was then added to the homogenate until 1 ml contained 25--30 mg of tissue. Next, 1 ml of homogenate or 0.5 ml of serum was transferred to a titration vessel in which was placed 25 ml of buffered solution. When titrating the SH- groups with $1 \cdot 10^{-3}$ M $AgNO_3$ solution we used a buffered solution containing 0.01 M NH_4OH and 0.08 M NH_4NO_3. When titrating the SH- groups with $1 \cdot 10^{-3}$ M $HgCl_2$ solution, we used a 0.1 M phosphate buffer (pH = 7.34), containing 0.05 M KCl. The method of titration was fundamentally identical with that described in the paper by Kolthoff and Harris [7]. The current in the circuit was recorded by means of a type M-91 microammeter with a sensitivity of 10^{-7} amp/ deg. The rate of revolution of the platinum electrode was 750-800 rpm. Titration was carried out in aerobic conditions. The titer of the $AgNO_3$ solution was checked by Mohr's method, and the titer of the $HgCl_2$ solution by a gravimetric method based on precipitation of mercury with H_2S. All the solutions were made up in double distilled water, redistilled from a glass vessel.

EXPERIMENTAL RESULTS

In the Table are shown the results of the experiments (each figure therein presents the average of 2-3 parallel determinations).

We see that the results of argento- and mercurimetric determination of the content of SH- groups in the

serum and brain homogenate are in practically complete agreement. It can be concluded from these results that during titration of SH- groups with silver nitrate in a mixed ammoniacal buffer, no significant oxidation or "disappearance" of these groups evidently takes place by the action of ammonium ions, since titration with $HgCl_2$ in a phosphate buffer gives the same results. The agreement of the results of argento- and mercurimetric determination of SH- groups also indicates that one mole of SH- groups in the serum and brain homogenate during titration in a phosphate buffer containing KCl. This conclusion is in full agreement with the results of the above-mentioned investigations by Kolthoff, Stricks and Morren [9].

Comparison of the Results of Argento- and Mercurimetric Titration of SH- Groups in the Serum and Homogenate of the Cerebral Cortex of the Cat

Experiment No.	Content of SH-groups in μM	
	in 100 ml of serum	
	$AgNO_3$	$HgCl_2$
1	61,5	63,0
2	47,5	45,0
	in 100 mg of brain tissue	
3	1,20	1,10
4	0,95	0,93

In conclusion it should be mentioned that the amperometric titration of SH- groups in the serum and especially in a tissue homogenate with silver nitrate has, in our opinion, a number of advantages over titration with mercuric chloride. In the first place, in titration of the SH- groups with $AgNO_3$ the value of the current is more rapidly established in relation to a given quantity of added reagent. In the second place, titration with $AgNO_3$ can be done in the presence of ethylenediaminotetraacetic acid (EDTA), which has the property of stabilizing the SH- groups of thiol compounds. From our findings, the content of SH- groups in tissue homogenate to which EDTA was added in a concentration of $3 \cdot 10^{-5}$ M does not vary over 14 hours when stored at a temperature of about 0°C. In the same homogenate not containing EDTA, 11-12% of the SH- groups is oxidized during storage for 24 hours under the same conditions.

SUMMARY

A simultaneous titration of SH-groups by $HgCl_2$ in phosphate buffer was undertaken for the determination of the question whether a noticable oxidation ("disappearance") of SH-groups takes place in the blood serum and in the brain homogenate under the effect of ammonium ion in their titration by $AgNO_3$ in diluted ammoniac buffer. Experiments demonstrated that the results of the argento- and mercurimetric determination of the concentration of SH- groups in the blood serum and brain homogenate coincide completely. These data permit to conclude that in titrating the SH-groups by $AgNO_3$ in diluted ammoniac buffer there is no appreciable oxidation or their "disappearance" under the effect of ammonium ion.

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