TIME COURSE OF ACTIVITY OF SODIUM AND HYDROGEN IONS AS CRITERIA FOR DETERMINING THE TIME OF DEATH

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To measure the activity of ions in biological systems the direct potentiometric method of analysis is nowadays widely used [1, 4]. This method has qualitative advantages over colorimetric analysis, flame photometry, complexometry, and other chemical methods that are difficult to perform [3]. Biological systems are very complex in composition, for they are mixed solutions of electrolytes. That is why highly selective electrodes are needed to study activity of a concrete ion [4].

In the investigation described below the relationship between the time after death and quantitative changes in free Na^+ and H^+ ions in the blood, cerebrospinal fluid, and vitreous body was studied in cadavers of experimental animals (rabbits) in order to obtain criteria enabling the time of death to be determined.

EXPERIMENTAL METHODS

Experiments were carried out on 42 cadavers of Chinchilla rabbits weighing 4-5 kg, kept until collection of the material at an air temperature of 17-20°C and relative humidity of 40-60%.

Blood for investigation was taken from the right subclavian vein. Serum was obtained by centrifugation at 1000g for 10 min. Cerebrospinal fluid (CSF) for investigation was taken from the cisterna magna. After collection the CSF was centrifuged to remove any blood or tissue cells accidentally contaminating it. The vitreous body was removed from the right eye by puncture at the external angle with a needle and the required volume of fluid was withdrawn by a syringe [2].

The concentration of Na^+ ions was determined by the method of standard additions, assuming known steepness of the electrode S function [3]. For the investigation 2.9 ml of a standard solution containing 50 mM NaCl, 10 mM KCl, and 70 mM Tris-HCl, pH 7.4, was poured into the cuvette. The ion-selective glass sodium electrode and the comparison electrode were placed simultaneously in the standard solution. With constant stirring on a magnetic mixer, the potential E_1 was measured. Next, 0.1 ml of the test fluid was added to the cuvette and the electrode potential E_2 was measured. Activity of Na^+ ions was measured 3 times and the result was recorded as the mean. The concentration of ions to be measured in the sample was calculated by known equations [3].

Activity of H^+ ions in blood, CSF, and vitreous body was determined by means of an apparatus with a range of sensitivity from 0 to 14.0 pH units and accuracy of determination of ±1% (from "Radiometer," Denmark). With this apparatus only 30 µl of liquid is required for the investigation.

The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The initial level of Na^+ ion activity in the blood (0 h) was 146.12 ± 1.42 mM (Fig. 1). Na^+ activity 2 h after death rose to 150.86 ± 2.14 mM and then showed a tendency to fall between 2 and 48 h, to reach 84.61 ± 2.64 mM by 48 h. Na^+ ion activity in the CSF in the ini-

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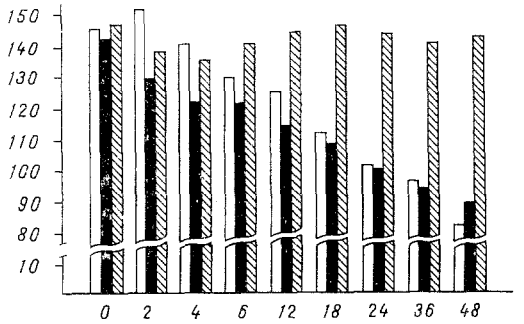

Fig. 1. Time course of Na^+ ion activity in blood, CSF, and vitreous body depending on time elapsing after death. Abscissa, time after death (in h). Ordinate, level of activity of Na^+ ions (in mM). Unshaded columns — blood, black columns — CSF, obliquely shaded columns — vitreous body.

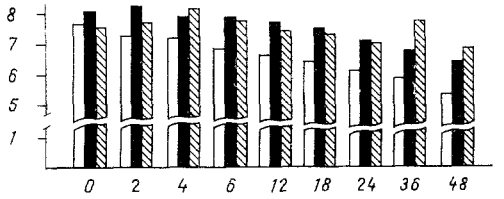

Fig. 2. Time course of H^+ ion activity in blood, CSF, and vitreous body depending on time elapsing after death. Ordinate, level of H^+ ion activity (in mM). Remainder of legend as to Fig. 1.

tial state was 142.98 ± 2.10 mM; later values were 129.85 ± 2.82 mM after 2 h, 121.52 ± 5.59 mM after 4 h, 122.42 ± 3.21 mM after 6 h, and after 12-48 h Na^+ activity fell gradually to reach 89.01 ± 2.72 mM. In the vitreous body at these same times Na^+ ion activity measured between 2 and 48 h after death fell during the first 2-4 h and then returned to normal.

Activity of H^+ ions in these same objects increased with an increase in the time elapsing after death (Fig. 2). For instance, in the initial state H^+ activity in the blood was 7.62 ± 0.06 pH units, falling to 5.87 ± 0.04 pH units after 48 h. At these same times H^+ ion activity in the CSF changed from 8.08 ± 0.08 to 6.99 ± 0.03 pH units, whereas in the vitreous it was between 7.58 ± 0.05 and 7.51 ± 0.34 pH units.

The use of Student's t test to determine statistically significant differences between the parameters showed that changes in Na^+ ion activity in the blood between 2 and 6 h ($P < 0.01$), between 12 and 18 h ($P < 0.01$), and between 36 and 48 h ($P < 0.01$) after death and in the CSF between 0 and 2 h ($P < 0.01$), between 6 and 12 h, 24 and 36 h, and 36 and 48 h ($P < 0.05$) were the most informative. In the vitreous body no statistically significant differences were found at any of these times.

The study of H^+ ions showed statistically significant differences in the blood between 0 and 2 h ($P < 0.01$), 4 and 6 h ($P < 0.05$), 6 and 24 h ($P < 0.01$), and 36 and 48 h ($P < 0.001$), in the CSF between 12 and 18 h ($P < 0.05$), 18 and 24 h ($P < 0.001$), 24 and 36 h ($P < 0.05$), and 36 and 48 h ($P < 0.001$). Values of H^+ ion activity in the course of investigation of the vitreous body did not change significantly.

The experimental data on the time course of changes in Na^+ and H^+ ion activity obtained in this investigation thus demonstrate that this method can be successfully used on autopsy material for the drawing up of expert criteria for determination of the time of death.

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