In previous communications [2, 3] we showed that other methods besides enzymic hydrolysis exist for the inactivation of acetylcholine (AC) in the organism, one of which is its fixation by elements of the circulating blood. The phenomenon of AC fixation has been studied by us in a large number of clinically healthy persons and also in patients with various forms of nervous diseases.

It has been found that the proportion of 1 ml of AC added in vitro (10−6), fixed by blood taken from healthy persons during storage for 24 h in a refrigerator, varies from 9 to 49% (mean 29 ± 13%), and the corresponding figures when the blood is taken from patients with a lesion of the diencephalic region are from 70 to 97% (84 ± 8%) and from patients with myasthenia—from 82 to 100% (90 ± 5%).

The results obtained during the study of the phenomenon of AC fixation in patients with other nervous diseases range from 0 to 95%. The mean value differs in each group of patients and depends to a certain degree on the tone and reactivity of the sympathetic and parasympathetic divisions of the autonomic nervous system. It has also been established that the AC fixed by blood may be restored to a free state (reactivation of AC) by incubation for 3 h at 37°.

We have postulated that the fixation of AC by the blood is a compensatory mechanism directed towards inactivation of the surplus of distant-acting AC in the fluid media of the organism. The phenomena of the fixation of histamine and serotonin (histamino- and serotoninoceptive effects) have been described in the literature [1, 4, 5, 6]. The existence of a bound form of AC in the organs and tissues has been known for some time, but there is no information regarding the fixation of AC by the blood.

Continuing our investigations, we have studied the mechanisms of the phenomenon of AC fixation and attempted to discover ways of influencing this process.

**EXPERIMENTAL METHOD AND RESULTS**

In the first place we attempted to determine whether the AC fixation in each individual subject is constant, or whether it may change as a result of various therapeutic procedures. Repeated investigations on healthy persons and patients at intervals of 2-3 months gave uniform results. Neither sex nor age differences could be detected. Consequently, the magnitude of the AC fixation is an individual characteristic of the organism, and is not dependent on change fluctuations in the neurohumoral regulation of functions. As therapeutic procedures, roentgen-ray irradiation of the thymus and operative removal of the gland were performed on patients under the care of L. B. Perel’man. The phenomenon of AC fixation was investigated before and after the course of treatment.

It was found that during a clinical improvement in the state of the patient the AC fixation by the blood falls to the level observed in healthy persons. Only in one case, when no clinical improvement was observed, the AC fixation fell insignificantly after thymectomy. In three patients with myasthenia, investigated once only after thymectomy, the fixation did not exceed 47%, i.e., it lay at the upper limit of normal, whereas very high fixation was characteristic of myasthenia. Hence, with an improvement in the myasthenic patient’s condition, the fixation as a rule returned to normal.
To elucidate the mechanism of AC fixation, 5 series of experiments were conducted.

1. Relationship between Fixation Effect, AC Concentration, and Blood Dilution

To two samples of blood from the same subjects AC was added in concentrations of $1 \times 10^{-8}$ (sample 1) and $1 \times 10^{-9}$ (sample 2). In all subjects the percentage of AC fixed in sample 2, in which its concentration was lower, was greater than that in sample 1. Consequently, with a reduction in the amount of AC added, a proportionally greater part of it passed into the bound state.

Simultaneously with the determination of the fixing power of whole blood, investigations were made of the fixation of AC by blood diluted with Ringer’s solution in proportions of 1:1, 1:2, and 1:3, the concentration of added AC being constant. It was found that the fixation fell in a linear relationship to the dilution of blood.

2. Importance of Individual Blood Elements for Fixation of AC

The fixing power of the erythrocytes, plasma, and hemolyzed erythrocytes was studied. For this purpose patients with a relatively high initial AC fixation were selected and the fixation of AC by centrifuged and washed erythrocytes, plasma, and erythrocytes hemolyzed with distilled water was investigated (Fig. 1). The fixation of AC by the erythrocytes was the same as its fixation by whole blood. After incubation, AC fixed by erythrocytes changed to the free form. The plasma of the same patients did not fix AC. Hemolyzed erythrocytes from patients with a high initial AC fixation in whole blood also failed to fix added AC.

3. Effect of Potassium Ions on Fixation of AC by Whole Blood and Washed Erythrocytes

Since potassium is known to influence certain stages of AC metabolism, for instance by increasing the ratio $\frac{\text{free AC}}{\text{fixed AC}}$, we studied the AC fixation phenomenon after addition of 0.1 ml of 2% KCl solution (2 ml to 8 ml of blood)