that prolonged barbiturate treatment results in a decrease of cholinesterase activity in serum and other tissues of mammalian subjects *in vivo,* has been confirmed in our *in vitro* studies with insect cholinesterase. Our studies on the changes in levels of the Ach-like substance in the insect nervous system during the course of poisoning by the chlorinated hydrocarbon insecticides show significant increases of this substance and also suggest a potentiation of the cholineacetylase system during such poisoning. Taking these facts into consideration, it may be suggested that the efficiency of the barbiturates as anticonvulsants depends, if not entirely, at least mainly, on their blocking of the action of Ach. The fact that withdrawal of barbiturate treatment may often give rise to *de novo* convulsions, may be the result, after metabolism of the barbiturate, of the partial destruction or inhibition of cholinesterase by the active barbiturate at its site or sites of action.

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Electron Microscopy of Human Erythrocytes from Healthy and Sludged Blood[1]

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Microscopic observation of the circulating blood of man, ill with a variety of disease processes, has shown that the red blood cells were struck into aggregates which varied in size and rigidity (*1–3*). In many diseases where tough red cell aggregates were seen *in vivo,* and coatings were presumed to hold these aggregates together, such coatings could not be demonstrated *in vitro* by light- or dark-field microscopy. Electron microscopy was undertaken to obtain better definition of the surface of the agglutinated red cells and thus to be able to demonstrate the morphology of the coatings.

The blood vessels of the human bulbar conjunctiva were obliquely illuminated, and a binocular biobjective microscope was focused on the circulating blood, which was studied at 48–150 diameters magnification (*4*). The results of the observations were recorded on a special chart.

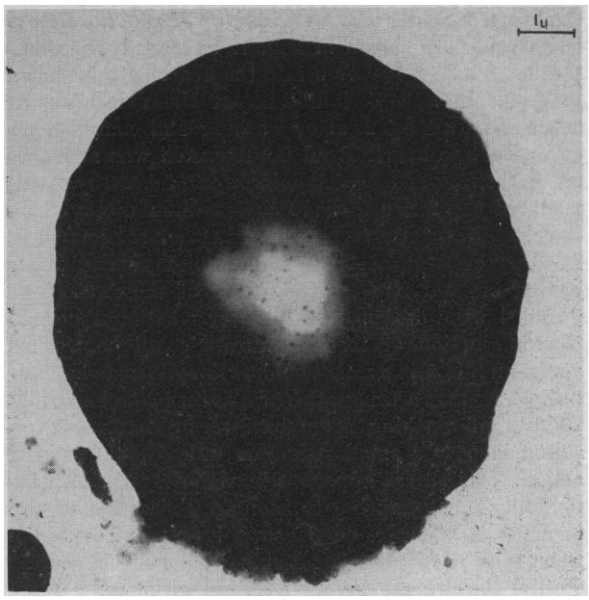

FIG. 1. Electron micrograph of a human red blood cell obtained from a healthy circulation. ×2000. Salt crystals are stuck to the lower border of the cell and can also be seen through center portion.

Two groups of subjects were used. In the first group were those who had a healthy circulation (*2*). This kind of circulation was demonstrated in clinically healthy subjects, as well as in those who were clinically ill but had a disease process which did not produce a sludge, or those who had had a pathologic circulation but had been treated and a normal circulation had been re-established. In the second group of patients were those who were clinically ill and had definite intravascular pathology characterized by the presence of a sludge which significantly reduced flow through arterioles. Such patients often had red cell aggregates with visible coatings.

Each patient's circulation was studied 24 hr before and immediately prior to the withdrawal of the blood for electron microscopy. The blood was obtained from the median cubital vein of the arm and was drawn directly into heparin, with the ratio of blood to heparin varying from 1:1 to 1:6. After withdrawal the blood and heparin were gently agitated in the syringe, and then 0.2–1.0 ml of the mixture was slowly ejected into 8–10 ml of mammalian Ringer's solution.

Electron microscopy of such blood was usually made within a period of 30–60 min after withdrawal. The diluted blood was prepared for electron microscopy by placing a small drop of the mixture upon a Parlodion film, which was placed on a 200-mesh screen. After 2 min the blood droplet was removed by absorption into filter paper, and the film with adhering red blood cells was air-dried and placed on the object holder. The

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preparations were studied on the viewing screen of an RCA microscope, and selected cells were photographed at magnifications ranging between 3,500 and 20,000 diameters.

Red blood cells from individuals having a healthy circulation were found by electron microscopy to have surfaces which had a sharp outline where no detail could be observed (Fig. 1). Such red blood cells were also found in small number in the blood of subjects who had sludged blood, where they were intermingled among the pathologic cells.

The blood obtained from patients who had sludged blood revealed red cells and/or red cell aggregates which had significantly altered surfaces. These red cells had a coating which covered the entire cell surface, was irregular in outline, varied in thickness, and was relatively amorphous in structure at magnifications up to 20,000 diameters (Fig. 2). Further, it was

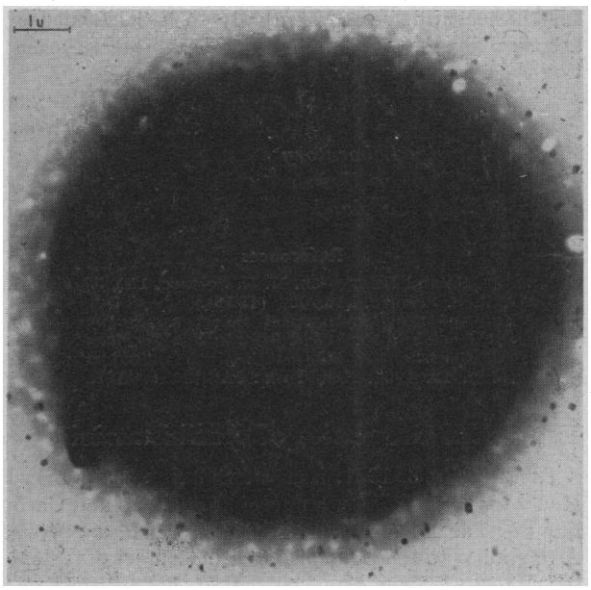

FIG. 2. Electron micrograph of a human red blood cell obtained from sludged blood (bronchopneumonia). ×2000. Note total increase in diameter of cell because of the coating, as compared with healthy cell of Fig. 1.

possible to obtain clumps of red cells and to demonstrate coatings not only covering the individual cells but also forming bridges between them (Fig. 3).

Since the demonstration, beginning in 1940, of sludged blood in human diseases, evidence has accumulated by various tests showing that the surfaces of the red cells of sludged blood are different from those of healthy blood. From direct microscopic observation of the living circulation came the evidence that the red cells of sludged blood were coated and were not infrequently bound together firmly by an appreciable amount of "new" substances of unknown composition. It was possible to demonstrate in the malaria of rhesus monkeys, *in vitro,* by dark-field microscopy, that the red cells of this sludge were held firmly together by a highly refractile substance. And it was possible to microdissect this coating and draw it into long, highly refractile, viscous filaments (*5*).

Although in the human diseases it has not hitherto been possible to demonstrate *in vitro* coatings on the

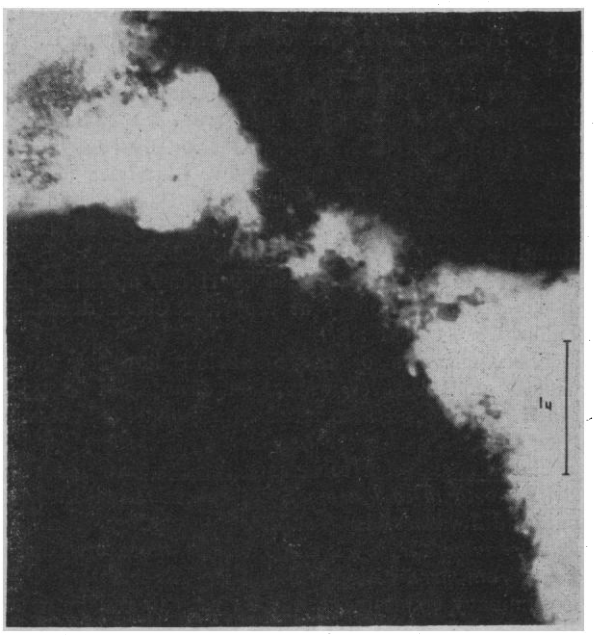

FIG. 3. Electron micrograph showing coating between two human red cells from sludged blood. ×5625.

red cells of sludged blood, it has been repeatedly demonstrated that the electrokinetic properties of these cells differed from the red cells of healthy blood (*6, 7*).

The demonstration of a coating on human red cells and aggregates by electron microscopy of sludged blood and of the absence of such coatings on the similarly treated red cells from healthy blood is in accord with and further buttresses the existing data that a marked change occurs on the surface of red cells in some diseases. Electron microscopy has not only given the first *in vitro* evidence of a coating on the human red cells from sludged blood but has also permitted a study of the coating's morphology.

Whether such coatings are derived principally from the cell or plasma still remains unanswered. Work is now in progress to characterize further the physical and chemical properties of the coatings on the red cells in human diseases.

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