Results. The experiments are summarized in Table I. The pulmonary edema produced by brain trauma (Exp. 2) is very striking. The absence of the adrenals (Exp. 3) failed to influence it.

Narcosis produced by various agents (Exp. 4, 5, 6) protected against the development of pulmonary edema. Adrenergic blocking agents (Exp. 7, 8, 9, 10) were also effective in varying degree.

Discussion. As has been noted above, the occurrence of other types of experimental pulmonary edema is also inhibited by adrenergic blocking agents(10,11,12). It has likewise been reported that narcosis prevents experimental pulmonary edema due to epinephrine in some species(14) inhibits ammonium pulmonary edema(11) and confers the best protective effect against the lung edema due to massive intracarotid infusions of saline(15). There is every indication that these various types of pulmonary edema are fundamentally similar in their origin and result from nerve stimuli of central origin, *i.e.*, they are all types of neurogenic pulmonary edema. The mechanisms through which these stimuli produce the edema is less clear. Visscher's group has stressed(16) the acute bradycardia and rise in pulmonary venous pressure associated with pulmonary edema. They find(16,17) that these changes precede the pulmonary edema due to increased intracranial pressure. The latter is essentially the same as the pulmonary edema which is produced more rapidly by traumatic injury of the brain. Campbell and Visscher(17) report that bilateral cervical vagotomy performed immediately beforehand in guinea pigs prevents or reduces the degree of lung edema due to increased intracranial pressure. We have examined the effect of this in brain trauma in guinea pigs and it is true that the lung edema is less severe. The most noticeable effect is the failure of a bradycardia to ensue. A tachycardia may even result. However bilateral cervical vagotomy alone eventually leads to the development of pulmonary edema and the problem appears to be not so simple as a question of efferent sympathetic stimuli from the brain which may be reduced by narcosis or adrenergic blockade and prevented by interrupting their flow through section of the vagi.

Summary. Experimental pulmonary edema occurs in the rat in a matter of seconds as a result of a blow to the calvaria causing fatal trauma to the brain. This edema is prevented or reduced in degree by agents which produce a deep narcosis and by adrenergic blocking compounds. The mechanism of its production is discussed in relation to other types of experimental pulmonary edema.

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Prolytic Changes in Chicken Erythrocytes Exposed to *Micrococcus aureus* Toxins.* (18019)

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Bernheimer(1,2) exposed human erythrocytes to the toxin of *Clostridium septicum* for

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20 minutes or more and then removed the toxin. He postulated that the toxin, possibly acting catalytically, combined with the erythrocyte resulting in an altered cell which swelled and then hemolyzed. These observations confirmed those of Menk(3) and conform to the hypothesis proposed by Wilbrandt (4) who suggested that some lysins increase the cation permeability of the erythrocyte which leads to osmotic hemolysis (*cf.* Davson, 5). In the present investigation chicken erythrocytes were exposed for a short period of time to a dilute toxin, which at higher concentrations had been shown to be hemolytic (6), and the toxin was then removed by washing. Such experiments should give some information concerning the interaction of toxin and cell. In addition, by using a concentration of toxin which produced hemolysis slowly, a long period of time was available for studying prolytic changes in the cells.

Materials and Methods. Four stock suspensions were made as follows: Equal volumes of chicken erythrocytes (obtained from heparinized blood) and Ringer Locke were placed in 2 vials and equal volumes of erythrocytes and a 1:100 dilution in Ringer Locke of *Micrococcus aureus* toxin (15,000 dermal necrotic doses/cc) were placed, in 2 other vials. These 4 vials were immediately put into a water bath at 37°C and kept at this temperature except for brief intervals required for centrifugation and other manipulations described below. After 10 minutes the contents of one control vial (A) and one experimental vial (B) were centrifuged, the supernatant fluids discarded and the cells resuspended in 10 cc of Ringer Locke. This gave a control (A) and an experimental (B) in which the toxin had been removed and the cells resuspended in Ringer Locke and a control (C) and an experimental (D) which had not been centrifuged. After various periods of incubation at 37°C the following observations were made: (1) A and B were centrifuged and the amount of hemoglobin in the supernatant fluid was measured by determining the percent transmission at 545 mμ using a Coleman Universal Spectrophotometer. This series of observations indicated the progress of hemolysis in stock suspensions A and B. (2) The rate of swelling was measured using the photoelectric apparatus previously described(7). An aliquot (0.09-0.13 cc) of each stock suspension (the centrifugate of A and B; C, D) was added to 10 cc of a solution of 0.3 M glycerol in Ringer Locke in the chamber of the apparatus. A Kipp and Zonen galvanometer was used and records were obtained on 12 cm bromide paper. These observations indicated the change in osmotic behavior that had occurred in the cells. (3) The glycerol—Ringer Locke —cell suspensions were removed from the photoelectric apparatus, centrifuged and the amount of hemolysis determined spectrophotometrically. These measurements indicated changes in "fragility"‡ of the cells. (4) Fragility measurements were made by adding aliquots of suspensions A-D to a series of tubes containing dilutions of NaCl from 1-0%. Following equilibration at 37°C the contents of these tubes were placed in the permeability apparatus and a record obtained. (5) Rates of hemolysis in ethylene glycol and glycerol (0.3M) were measured using the photoelectric apparatus. (6) Equal volumes of the stock suspensions (A-D) and 0.3 M glycerol in Ringer Locke were mixed and left at 37°C for at least 10 minutes. The rate of shrinking was recorded by adding an aliquot of these new suspensions (A'-D') to 10 cc of Ringer Locke in the permeability apparatus. This technic, described by Wil-

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‡ The quotation marks are used to indicate that a different type of measurement is involved in obtaining these values. In measuring fragility cells hemolyze as a consequence of the hypotonicity of a salt solution. In measuring "fragility" the cells hemolyze as a consequence of shrinking and swelling to their initial volume(8).

TABLE I.

A typical experiment indicating the progressive hemolysis which occurred in washed chicken erythrocytes which had been exposed to the toxin of *M. aureus* for 10 min. at 37°C. Data were obtained spectrophotometrically and compared with a standard hemolysis curve. The values indicate the amount of hemolysis which had occurred since the preceding measurement while standing at 37°C.

	% hemolysis	
Time in hrs	Control (A)	Experimental (B)
½	1	< 1
5	< 1	< 1
11	< 1	1
27½	4	9
53	11	>25

TABLE II.

A typical experiment indicating the progressive increase in "fragility" (measured spectrophotometrically as hemolysis occurring in a solution of 0.3 M glycerol in R.L.) of chicken erythrocytes exposed to the toxin of *M. aureus* (one pair washed (A,B), the other pair unwashed (C,D)).

	% hemolysis*			
Time in hrs	A	B	C	D
½	1.6	1.6	1.3	0.8
14	2.5	3.8	2.0	7.8
23½	2.5	2.5	3.5	12.2
41	3.5	3.5	3.8	29.4
61	3.8	11.8	13.1	40.5

* These values include the hemolysis which occurred in the stock suspension prior to shrinking and swelling.

brandt(9), gives a measure of permeability to supplement the swelling data. (7) These Ringer Locke suspensions were centrifuged following (6) above and the percent of hemolysis was determined spectrophotometrically. This gives additional information concerning the fragility of the cells.

Following these observations, 10 cc of Ringer Locke were added to A and B and they remained at 37° until some later time when the above observations were repeated. Bacteriologically sterile technics were used except for the few minutes required to make the actual observations. Sterility tests were made as previously described(10).

Results. Table I shows the progressive hemolysis in A and B. As one might expect, the control cells hemolyzed slowly over a period of many hours while the cells which had been exposed briefly to the dilute toxin hemolyzed a little more rapidly. Table II indicates what percent of the cells hemolyzed when allowed to shrink and swell in a hypertonic solution of a penetrating molecule in a balanced salt solution. As indicated by D, the toxin in this dilution affects the cells slowly, one of the prolytic changes being a progressive increase in "fragility" beginning about 12-13 hours after exposure to the toxin. Cells which have been exposed to the toxin for only 10 minutes (B) show a similar progressive increase in "fragility" but in general this seems to occur more slowly. A comparison between A and C shows no consistent effect of washing and centrifuging the cells on "fragility," although in most of the experiments the cells in A appeared to be more "fragile" (*cf.* Davson and Danielli,11).

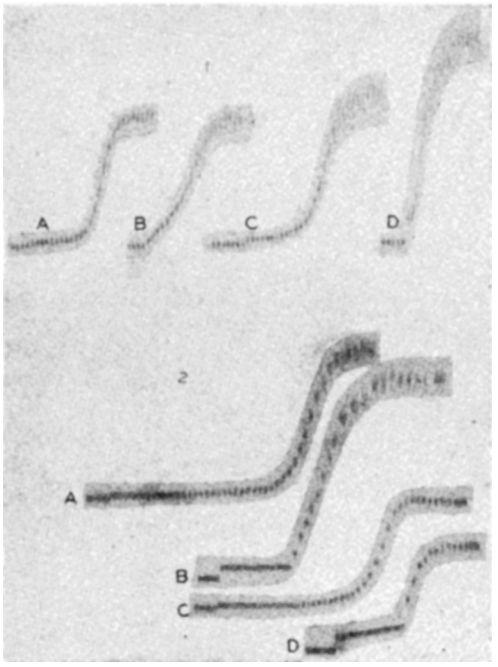

FIG. 1.

Effect of the toxins of *M. aureus* on the rate of hemolysis of chicken erythrocytes in 0.3 M ethylene glycol (1) and in 0.3 M glycerol (2). Time intervals: 1—sec; 2—first 15 sec. continuously, every 15 sec. A—washed control; B—washed toxin; C—unwashed control; D—unwashed toxin.

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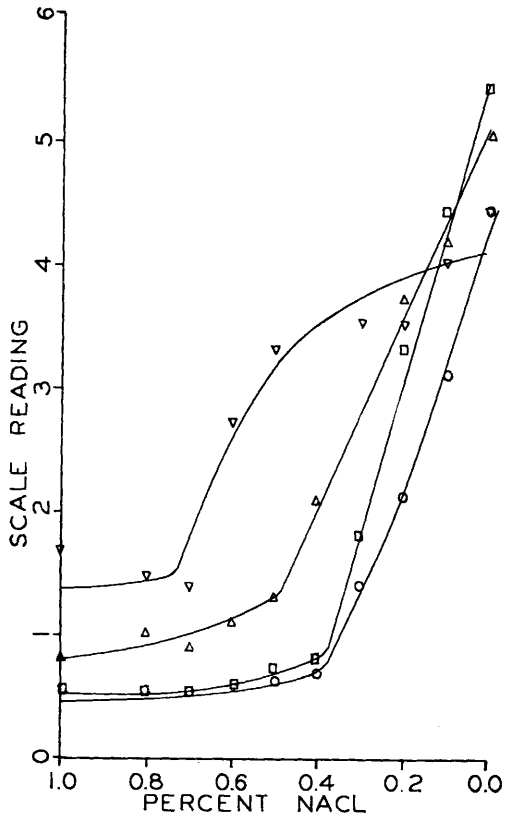

FIG. 2.
Effect of the toxins of *M. aureus* on the fragility of chicken erythrocytes. ○—washed control; (A); △—washed toxin (B); □—unwashed control (C); inverted triangle—unwashed toxin (D).

Fig. 1 shows hemolysis in ethylene glycol and glycerol. The time for hemolysis is essentially the same with the washed and unwashed control cells (there is some indication in most of the experiments that the washing altered A cells slightly), while the cells which had been exposed briefly to the toxin (B) hemolyzed more rapidly and those cells still in the presence of the toxin (D) hemolyzed most rapidly. The same sequence was observed when fragility was measured (Fig. 2). The cells in D were most fragile, those in B less fragile and those in A and C least fragile. Fig. 3 shows the swelling of cells in 0.3 M glycerol in Ringer Locke and the shrinking of cells which had been equilibrated in this solution and then placed in Ringer Locke. Spectrophotometric measurements which were made after the shrinking curves had been obtained gave respectively the following values for per cent hemolysis: A'—55%; B'—58%; C'—34%; D'—>> 60%. These curves actually give some indication of the fragility of the cells, for as they swell, some reach their hemolytic volume. This is indicated at the top of the curve just before shrinking begins. The hemolysis values for these solutions given above are an index of fragility. In the case of the cells in D, they were so fragile (Fig. 2) that no shrinking curve could be obtained for most of the cells hemolyzed during their initial swelling.

Some interesting comparisons can be made between the data obtained from cells exposed to the toxins for 40-45 hours that have been discussed above and cells exposed for 5-7 hours. In the latter case the fragility, swelling and shrinking curves obtained with A, B, C and D are all essentially identical. Also, the shrinking curves show less hemolysis just prior to shrinking. Spectrophotometer read-

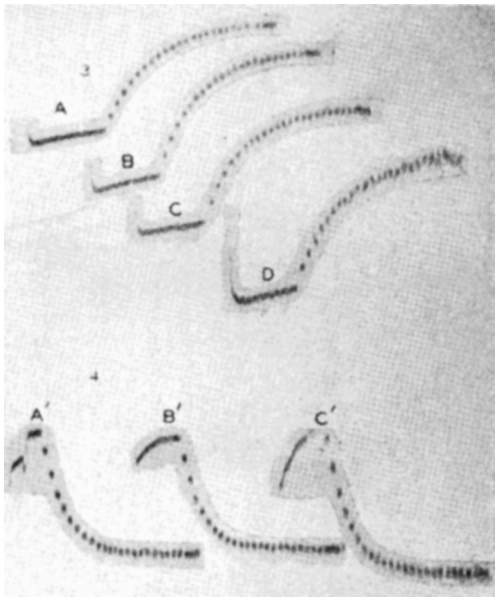

FIG. 3.
Effect of the toxins of *M. aureus* on the rate of swelling in 0.3 M glycerol in Ringer Locke (3) and the rate of shrinking in Ringer Locke following equilibration in 0.3 M glycerol in Ringer Locke (4). A,A' washed control; B,B'—washed toxin; C,C'—unwashed control; D—unwashed toxin.

11. Davson, Hugh, and Danielli, J. F., *Biochem.*, 1938, v32, 991.

ings showed the following per cents of hemolysis for swelling and shrinking: A—8%; B—4%; C—7%; D—10%; A'—7%; B'—4%; C'—6%; D'—6%. The second series of values are another indication of little if any fragility change while the first series shows little or no increase in "fragility." After this length of time, then, the toxin had had little effect on the cells.

Discussion. The prolytic changes observed in these experiments are an increase in the fragility (and "fragility") of the cells and a decrease in the time for hemolysis in solutions of lipid insoluble non-electrolytes. The rates of swelling and of shrinking, however, are not altered to any appreciable extent. This suggests that there is little if any change in permeability, the difference in rates of hemolysis being a consequence of the change in fragility of the cells. It is of interest to note that similar observations have been made on chicken erythrocytes exposed to the toxins of *B. cereus*(12) and *Cl. perfringens*(13).

A comparison of the washed and unwashed toxin-treated cells suggests that using toxin dilutions which are weakly hemolytic, some irreversible change in the membrane occurs very rapidly (less than 10 minutes). Since a number of workers(14-17) have suggested various mechanisms including adsorption, fixation or complex formation between lytic agents and erythrocyte membranes, it would seem reasonable to suggest that some toxin stays with the cells while that which is uncombined is removed by the washing. Since the unwashed experimental cells changed more rapidly than those which had been washed it can be assumed that a small, fixed quantity of toxin will alter the cells gradually, but if a "reserve" of toxin molecules is present, the change in the cells will take place more rapidly. This conclusion is consistent with observations such as those of Ponder(18,19).

Although the preceding studies using saponin(20) and toxins of *Str. β-hemolyticus* (21) did not involve as complete an analysis as in the present experiments, there is reason to believe that the prolytic action of these two substances does differ from the action of the toxins of *M. aureus*. This is not surprising for although Ponder and McLachlan(22) reported a similarity in the kinetics of hemolysis by saponin and toxins, Bernheimer(1) has shown that this is not the case.

Conclusions. 1. Chicken erythrocytes exposed to a dilute toxin obtained from *Micrococcus aureus* progressively become more fragile prior to hemolysis. 2. These cells hemolyze more rapidly in isosmotic solutions of ethylene glycol and glycerol. 3. Swelling and shrinking measurements show that the toxin has little or no effect on the permeability of these cells to glycerol. 4. The fact that more of the toxin-treated cells hemolyze following shrinking and swelling in 0.3 M glycerol in Ringer Locke than do controls suggests that the toxin may weaken the membrane in some way. 5. A comparison between erythrocytes exposed to the toxin for 10 minutes and then washed and erythrocytes continuously in the presence of the toxin indicates that the toxin effects an immediate and irreversible change. 6. Even though diffusible toxin is removed by washing, some toxin apparently remains and gradually the cells hemolyze. This process occurs more rapidly if the diffusible toxin is not removed.

12. Unpublished results.

13. Unpublished results.

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