pH alters the charge density of fibrinogen as the isoelectric point is approached. Results of preliminary studies in which red cells were suspended in a chelating medium (EDTA), however, tend to be supportive of the diffuse cationic layer concept presented above.

Summary. The amount of fibrinogen adsorbed from plasma by erythrocytes varies with the pH and inversely with the pCO_2. The influence of pCO_2 is probably mediated through its alteration of blood pH. The influence of pO_2 on erythrocytic adsorption of fibrinogen is negligible. Physiological alterations in pH may affect the amount of fibrinogen stored on erythrocytic surfaces or released to the plasma compartment.

1. Traber, D. L., Kolmen, S. N., Tex. Rep. Biol. Med., 1965, v23, 782.
2. Traber, D. L., Haynes, J., Daily, L. J., Kolmen, S. N., ibid., 1963, v21, 587.
3. Kolmen, S. N., Maynes, C., ibid., 1965, v23, 844.
4. Abramson, H. A., Moyer, L. S., J. Gen. Physiol., 1936, v19, 601.
5. Abramson, H. A., J. Exp. Med., 1927, v46, 987.
6. ———, J. Gen. Physiol., 1931, v14, 163.
7. Seegers, W. H., Nieft, M. L., Vandenbelt, J. M., Arch. Biochem. Biophys. 1945, v7, 15.
8. Astrup, T., Piper, J., Acta Physiol. Scand., 1946, v11, 211.

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Effect of Age on Surface Properties of Excised Lungs.* (31142)

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It has been reported that excised lungs subjected to numerous cycles of inflation and deflation in rapid succession lose their normal stability of expansion(1). This was readily confirmed in adult rats, but infant lungs failed to show the change. The present study was undertaken in order to examine the effect of repeated inflations on stability of expansion in relation to age.

Material. All lungs from cats, dogs and rabbits were apparently normal. The numbers and ages are indicated in Fig. 1. Of the 7 adult rats examined, 4 had an initial stability within the normal range, and were used in arriving at the data in Fig. 1. The remaining 3 rats had an abnormally low initial stability. This is known to occur especially among rats more than 6 months old and has tentatively been ascribed to early chronic murine pneumonia(2). The human material, derived from autopsies on infants, is quite heterogeneous (Fig. 2). Among neonates, both above and below a body weight limit of 1000 g, a large proportion were clustered in a high-stability area, and a similar number had very low stability. Only the high-stability cases were used to construct the line for newborns in Fig. 1. The low-stability cases as well as the majority of an intermediate group (Fig. 2b) may be assumed to have had the respiratory distress syndrome. All those who survived more than 2 hours after birth, had hyaline membranes and the others would presumably have developed them had they survived longer(3). One infant with high stability and hyaline membranes was presumably recovering from the pulmonary manifesations of the disease when it died at 2 days of age(3). There is a lack of older infants in the present study, and therefore initial stability data obtained by a few, slow inflation cycles, are added to Fig. 2c and denoted by circles.

Method. Specimens from infants, usually the left lung, were obtained at autopsy and inflated with the apparatus previously described. This is based on the method of Gribetz, Frank and Avery(4) in which the amount of air displaced from a reservoir into the lung is measured with a burette. The desired pressure is produced by raising the burette, and read on a water manometer. Separate lobes of lungs of small adult dogs

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were similarly treated. (The capacity of the equipment available was limited to about 200 ml.) All other animals were killed by intraperitoneal injection of pentobarbital sodium (Nembutal). Using the same apparatus, the lungs were examined in the intact chest (except for openings cut in the diaphragm) because removing them from the chest frequently caused leaks. The highest pressure used was 50 cm of water for human infants, and 40 cm for animals. The volume was read at that level and on deflation to 20, 10, 5 and 0 cm. The stability index was calculated by a method previously described (5), relating the volume remaining at deflation to 10 and 5 cm, to the maximal volume at 40 or 50 cm: $(2V_5 + V_{10})/2V_{max}$. Repeated expansion and deflation was carried out by rapidly raising and lowering the burette. The speed was probably limited by the movement of water in the apparatus rather than the flow of air in and out of the lung. This varied from 8 per minute in the smallest specimens to 2 per minute in the largest, usu-

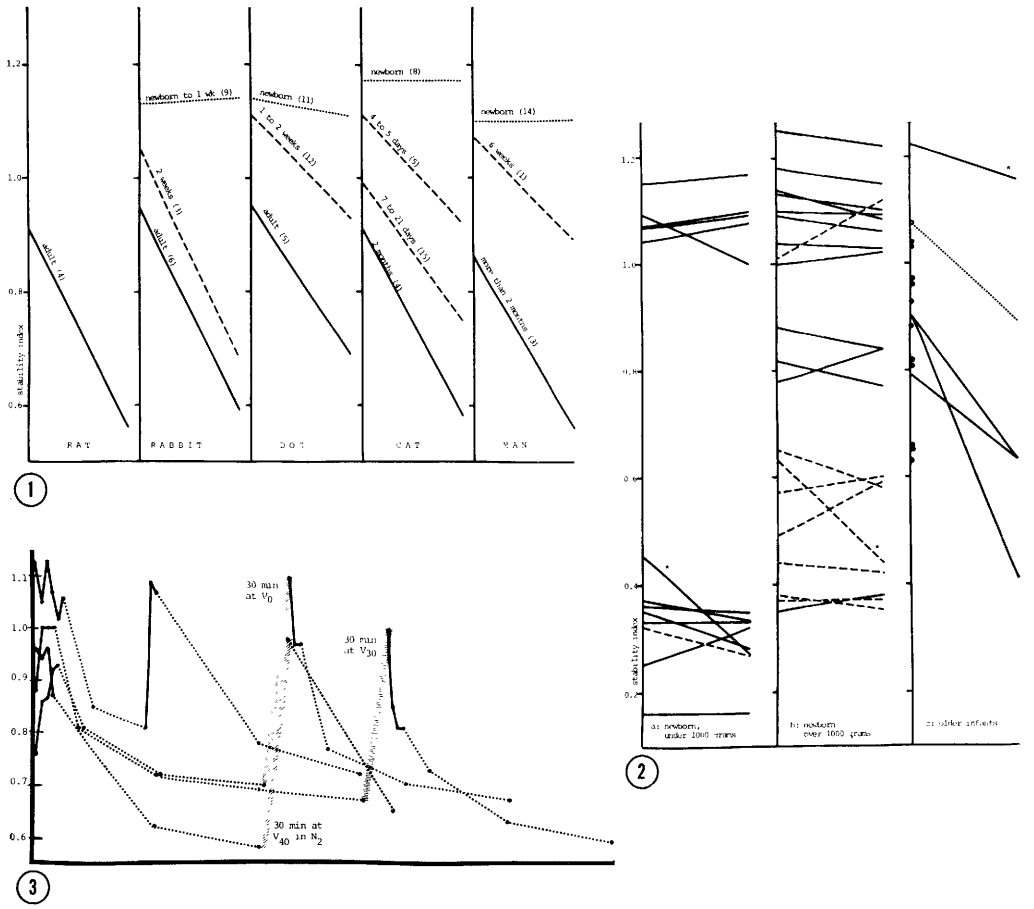

FIG. 1. Change in stability index of lungs with repeated inflation. Initial stability is along the vertical lines, and final stability after 20 to 100 inflations to the right. Numbers of specimens examined are in brackets.

FIG. 2. Change in stability index in lungs of human infants, drawn as in Fig. 1. Broken lines indicate lungs with hyaline membranes. In *c* the dotted line indicates a 6-week-old infant, all others are more than 2 months old. Circles indicate cases in which only initial stability was determined: *open,* 3 to 6 weeks; *solid,* 2 months and older.

FIG. 3. Change in stability index with repeated inflations, and recovery of lungs of 2-month-old cats. Number of inflations is along the horizontal axis; total length corresponds to about 100 cycles. Solid lines: change after single cycles; dotted lines: change after multiple cycles in rapid succession. Other shaded areas indicate condition tested for recovery as indicated.

ally 6 per minute. In contrast, one cycle with readings taken at intervals on inflation and deflation took about 5 minutes.

Some animal lungs were examined within a few minutes after death, others within a few hours and some were kept under refrigeration over night. In human material the body was refrigerated until permission for autopsy was obtained. This has no effect on the results(3).

It has been stated that recovery of stability upon standing is prevented by inflating lungs with nitrogen(1), and this was also tested. The air space of the apparatus was flushed with nitrogen for several minutes, and pyrogallol was added to the water to eliminate whatever oxygen might be present. This solution was never in contact with the specimen.

Since specimens could not be degassed, air content was estimated in some specimens at the end of the experiment by weighing, and then measuring the volume of the lungs by immersion in water. From this the content at the beginning could be estimated. It was never large enough to have a significant effect on the stability index.

Some decrease of stability could be detected after as few as 3 to 5 rapid cycles, and the greater part of the decline was accomplished after 20 rapid cycles. If no change was evident after 20 cycles, 20 to 80 additional cycles never produced an effect. Since even one slow cycle as used to obtain stepwise volume measurements results in partial recovery, the number of readings had to be kept to a minimum. At least one uninterrupted group of 20 cycles was carried out. Following this, readings were taken on deflation in the twentieth cycle.

Observations. The results are summarized in Fig. 1, showing the change of stability after 20 to 100 rapid expansions and deflations.

Neonates. The range of the age group varied from species to species: In the rabbit, 1-week-old specimens behaved like those of neonates and are included with them. This was not the case in the dog and cat. In man most specimens listed as normal newborns were less than 3 days old; only one, a 940-gram infant, was 12 days old. In all newborn groups the initial stability was high (index higher than 1.1) and showed no change after repeated expansion.

Adults. Specimens from adult animals were examined only in 3 of the species. The oldest cats were 2 months old, and the humans 2 months to 2 years. The stability of the latter 2 groups of specimens conformed to that of the adults of other species, and will therefore be considered along with those. The average initial stability in all groups was significantly below that of neonates, yielding an index in the vicinity of 0.9. Upon repeated inflation there was a marked and consistent drop of stability, in 4 of the groups to levels below 0.6 and in one to less than 0.7.

Intermediate ages. Young individuals were available as indicated in Fig. 1. In these groups the initial stability value was between those obtained in newborns and adults. In some instances the drop of stability upon repeated inflation, gradually matures at a rate differing from species to species.

Abnormal specimens. Three rats were encountered which had an abnormally low initial stability, tentatively ascribed to early chronic murine pneumonia(2). The loss of stability after multiple rapid inflations was of the same order as that in rats with higher initial stability.

Somewhat more than half the human neonatal specimens had an abnormal initial stability (Fig. 2). This is known to be associated with the respiratory distress syndrome. With 2 exceptions they also failed to show a change in stability as did the normal ones. In the majority of instances the initial stability was so low that one might suspect further lowering to be impossible or unlikely. There are, however, 2 intermediate groups including 3 cases with a stability index near 0.8, and 3 cases about 0.6, in which the index also remained essentially unchanged. Two cases showed a moderate, but definite drop in stability: a 600-gram stillborn (Fig. 2a,*), and a 1400-gram infant dying at 6 hours with hyaline membranes (Fig. 2b,*); this is unexplained. Another unusual case was a 64-day-old infant born at 28 weeks of gestation, weighing 1450 g at death. In spite of its advanced postnatal age, it conformed

to neonates with regard to initial and final stability (Fig. 2c,*). This is consistent with the supposition that conceptional rather than postnatal age governs maturation of the process here under consideration. However, one single case is insufficient to make a definite assertion.

Recovery of stability. It had been stated that following a decline in stability after repeated inflation, recovery occurs if the lung is kept inflated for some time with pure oxygen or room air but not with nitrogen or carbon dioxide(1). Therefore, the circumstances of recovery were investigated. Also, this became necessary when it turned out that minor delays in the course of an experiment interfered with the loss of stability. Almost complete recovery of stability of the lungs of 2-month-old kittens occurred if for a period of 30 minutes the specimen was kept inflated at a pressure of 30 or 40 cm with either air or nitrogen, or if the lung was left collapsed (Fig. 3). In many instances it was sufficient to follow rapid inflations with one 5-minute cycle of inflation and deflation (as is necessary to take 8 to 10 readings for a pressure-volume loop) to obtain recovery of stability (Fig. 3). Since minute details in performing experiments may profoundly affect the results, one cannot say that our results are in disagreement with those of Faridy *et al* (1), though they differ with regard to recovery after inflation with nitrogen.

Other variations of experiments. It had previously been shown that inflation at a temperature of 48°C lowers high, and raises low stability(6). The lungs of 4 newborn infants, some with good and some with poor stability, were repeatedly expanded at that temperature. There was no change in stability from the initial value at 48°.

In an effort to ascertain whether rapid inflation or rapid deflation is more effective in lowering stability, the lungs of 2 adult rats were repeatedly expanded slowly (about 30 seconds) and then deflated rapidly, and 2 other specimens were inflated rapidly and deflated slowly. The effect on stability was less than when both inflation and deflation were rapid, but no striking difference between the 2 variants of the experiment were found. However, slow inflation produced a higher residual volume.

Discussion. The situation in which changes of stability were examined differs fundamentally from the state during life. In the living there must obviously be a mechanism by which stability of expansion is maintained. The excised lung probably represents a state in which this mechanism no longer operates, although it has been asserted that the alveolar lining cells can still replace used-up surfactant in the excised lung kept at room temperature for several hours(1). It is likely that in the excised lung (and it made no difference whether examinations were performed shortly after death or after the specimen had been refrigerated for a day) a given amount of pulmonary surfactant is present which may undergo minor, partially or totally reversible changes in molecular structure or arrangement. The present observation in adult animals and older human infants suggests that restitution of the alveolar lining layer to its active state during deflation requires more time than was available during repeated inflation at a rate of a few cycles per minute. Restitution is accomplished to some extent in a few minutes; the exact time has not been ascertained. It is likely that this recovery is not complete, since subsequent repeated inflations may lower stability more rapidly than at the beginning of the experiment. Under the present conditions recovery occurred in the deflated state, or when the lung was kept inflated with room air or with nitrogen. This does not support the suggestion that the alveolar lining cells produce new surfactant by a process requiring oxygen(1).

Failure of neonatal lungs to lose stability upon repeated expansion may be due to quantitative or qualitative differences between the adult and the neonate in the active lining layer. This difference is of great magnitude: in the former a decline of stability is demonstrable even after 3 rapid cycles, whereas in the newborn no change occurs even after 100 cycles. In no instance did stability decline after continued cycling if there had been no change after 20 cycles. This suggests, but does not prove that a mere difference in the amount of surfactant is not the sole cause of

this divergent behavior. In this context observations in abnormal infant lungs are of interest. It has been suggested, and some evidence presented(7) that in very small premature infants and in the respiratory distress syndrome the amount of surfactant in the lungs is reduced. If quantity of surfactant were the cause of the differences observed here, then these particular lungs should lose stability more readily than any others. Yet this is not the case either in very small infants (Fig. 2a), or in those with the respiratory distress syndrome (Fig. 2b). This is additional evidence that a qualitative diference exists between the newborn and the adult which causes the alveolar lining layer of the newborn to preserve its surface activity after repeated cycling of the excised lung while this is not so in the adult. The transition is gradual since intermediate stages have been observed (Fig. 1). The cause of this difference in behavior and presumably structure of the surfactant is unknown. The amount of phospholipid in lung tissue of the ewe is about ⅔ of that in her full term fetus for unit dry weight(8), even though it may be assumed that a greater potential surface area is contained in the sample from the adult per unit weight. It is known that the fatty acid content of the pulmonary phospholipid affects its surface activity. Therefore, it is pertinent to note that differences in serum cholesteryl ester fatty acids have been found between newborn and adult rats(9). It remains to be determined whether differences of this kind also exist in the surface active pulmonary phospholipid.

Summary. Excised lungs of human infants and 4 animal species were inflated and deflated at 2 to 8 cycles per minute. This resulted in a loss of stability of expansion in all adult and post-neonatal infant specimens tested, but was not true in neonatal lungs. It is suggested on the basis of available data that a qualitative as well as a quantitative difference exists between newborn and adult lungs in the surface active material of the alveolar lining layer. Intermediate stages have been observed in post-neonatal infant lungs of all species. Recovery of stability of expansion occurred at rest within a few minutes regardless of the state of expansion, or the use of air or nitrogen.

1. Faridy, E. E., Johnson, J. W. C., Permutt, S., Physiologist, 1964, v7, 128.

2. Gruenwald, P., Am. J. Clin. Path., 1964, v41, 176.

3. ———, Arch. Path., 1965, v80, 30.

4. Gribetz, I., Frank, N. R., Avery, M. E., J. Clin. Invest., 1959, v38, 2168.

5. Gruenwald, P., J. Appl. Physiol., 1963, v18, 665.

6. ———, Arch. Path., 1964, v77, 568.

7. Adams, F. H., Fujiwara, T., Emmanouilides, G., Scudder, A., J. Pediat., 1965, v66, 357.

8. Tierney, D. F., Parker, N., Felts, J., personal communication.

9. Lopez-Santolino, A., Miller, O. N., Nuldrey, J. E., Proc. Soc. Exp. Biol. and Med., 1965, v118, 829.

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Rubella Virus: Growth Characteristics and Stability of Infectious Virus and Complement-Fixing Antigen. (31143)

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Rubella virus specific complement-fixing (CF) antigen was recently prepared by Sever *et al*(1) using the "packed cell" method with continuous rabbit kidney (RK-13),* primary African green monkey kidney (AGMK), as well as other tissue culture systems. The data

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